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(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1	2096 S MAPKAP##
L2	5 S L1 AND (MAPKAP-2 (W)KINASE?)
L3	4 DUP REM L2 (1 DUPLICATE REMOVED)
L4	119389 S PARATHYROID (W)HORMONE##
L5	6 S L1 AND L4
L6	5 DUP REM L5 (1 DUPLICATE REMOVED)
L7	221 S PARATHYROID (W)HORMONE-2
L8	1 S L1 AND L7
L9	68 S IMMUN? AND L7
L10	1 S L1 AND L9
L11	369433 S SKELETAL (W)MUSCLE
L12	387 S L4 AND L11
L13	0 S L7 AND L11
L14	76 S L7 AND (ISOLAT? OR PURIF?)
L15	33 DUP REM L14 (43 DUPLICATES REMOVED)
L16	87 S L7 AND (EXPRESS? OR EXCRET?)
L17	44 DUP REM L16 (43 DUPLICATES REMOVED)
L18	12 S L7 (2W)LIGAND?
L19	4 DUP REM L18 (8 DUPLICATES REMOVED)

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NEWS EXPRESS JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT  
MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),  
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\* \* \* \* \* STN Columbus \* \* \* \* \*

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=> s MAPKAP##

L1 2096 MAPKAP##

=> s l1 and (MAPKAP-2 (w)kinase?)

L2 5 L1 AND (MAPKAP-2 (W) KINASE?)

=> dup rem l2

PROCESSING COMPLETED FOR L2

L3 4 DUP REM L2 (1 DUPLICATE REMOVED)

=> d 1-4 ibib ab

L3 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:141038 HCAPLUS

DOCUMENT NUMBER: 142:240427

TITLE: 1H-indazole-3-carboxamide compounds as MAPKAP  
kinase modulators and their preparation

INVENTOR(S): Wyatt, Paul Graham; Gill, Adrian Liam; Saxty, Gordon;  
Apaya, Robert

PATENT ASSIGNEE(S): Astex Technology Limited, UK

SOURCE: PCT Int. Appl., 107 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005014554	A1	20050217	WO 2004-GB3388	20040806
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,			

EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,  
SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,  
SN, TD, TG

PRIORITY APPLN. INFO.: GB 2003-18719 A 20030808  
US 2004-543496P P 20040211

OTHER SOURCE(S): CASREACT 142:240427; MARPAT 142:240427

AB The invention is related to the use of compds. I and their salts, solvates (e.g. hydrates), and N-oxides [wherein A = a bond, CH<sub>2</sub>; R<sub>1</sub> = 3-12-membered carbocyclic or heterocyclic ring; R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub> = independently H, halo, OH, CF<sub>3</sub>, NO<sub>2</sub>, NH<sub>2</sub>, CN, etc.] in the prophylaxis or treatment of a disease state or condition mediated by a MAPKAP kinase. The invention is also related to the preparation of compds. I. For example, Pd-coupling of N-[4-[(methylaminosulfonyl)methyl]phenyl]-5-iodo-1H-indazole-3-carboxamide (preparation given) with furan-2-ylboronic acid gave 37% II. Selected I had IC<sub>50</sub> values < 150 µM or provided at least 25% inhibition of the MAPKAP K-2 kinase activity at a concentration of 100 µM.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:71068 HCAPLUS

DOCUMENT NUMBER: 142:150832

TITLE: Novel inhibitors of mammalian YAK3 and/or  
MAPKAP-2 kinases for  
treating various disorders

INVENTOR(S): Sato, Hideyuki; Takada, Mio; Washio, Yoshiaki

PATENT ASSIGNEE(S): Smithkline Beecham Corporation, USA

SOURCE: PCT Int. Appl., 52 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005007092	A2	20050127	WO 2004-US21701	20040707
WO 2005007092	A3	20050609		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

EP 1641766 A2 20060405 EP 2004-777657 20040707

R: LT, LV, HR

PRIORITY APPLN. INFO.: US 2003-485365P P 20030708  
WO 2004-US21701 W 20040707

OTHER SOURCE(S): CASREACT 142:150832; MARPAT 142:150832

AB This invention relates to newly identified inhibitors of YAK3 and/or MAPKAP-2 (MK2) kinases in mammal for treating various disorders, i.e.: neutropenia; cytopenia; anemias, including anemias due to renal insufficiency or to a chronic disease, such as autoimmunity or cancer, and drug-induced anemias; polycythemia; myelosuppression; rheumatoid arthritis; COPD; asthma; psoriasis; acute neuronal injury; heart failure; stroke, osteoarthritis; and ischemia reperfusion injury. Preparation of various inhibitors of YAK3 and/or MK2 kinases is described.

L3 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN



DUPLICATE 1

ACCESSION NUMBER: 2003-08555 BIOTECHDS

TITLE: New isolated nucleic acid molecule encoding a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2), useful for treating immune-system related disorders, inflammation and arthritis; recombinant enzyme protein production and sense and antisense sequence for use in gene therapy

AUTHOR: LOGRASSO P; HAWKINS J; LISNOCK J M

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002090524 14 Nov 2002

APPLICATION INFO: WO 2002-US5670 25 Feb 2002

PRIORITY INFO: US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-111970 [10]

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if its is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the

binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to subtracting the product from  $X_a$ . The nucleic acid molecule has a sequence that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a

dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay. In (M13), the allelic variant is encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound. (M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15),

identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

ADMINISTRATION - Administration is oral, topical or parenteral (e.g. intraarterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal). The dosage is 0.001-50 mg/kg, preferably 0.1-1.0 mg/kg.

EXAMPLE - No relevant example given. (150 pages)

L3 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2001:470402 HCAPLUS

DOCUMENT NUMBER: 136:83416

TITLE: Signal transduction in the adapted heart: Implication of protein kinase C-dependent and -independent pathways

AUTHOR(S): Debarros, John; Das, Dipak K.



CORPORATE SOURCE: Department of Surgery, University of Connecticut  
 School of Medicine, Farmington, CT, 06032, USA  
 SOURCE: Progress in Experimental Cardiology (2000),  
 3(Hypertrophied Heart), 3-16  
 CODEN: PEXCFF; ISSN: 1389-1774  
 PUBLISHER: Kluwer Academic Publishers  
 DOCUMENT TYPE: Journal; General Review  
 LANGUAGE: English

AB A review. Cardioprotection as a result of myocardial adaptation to cellular stress is a product of evolution. Myocardial adaptation potentiates intracellular signaling involving diverse signal transduction pathways. Ischemic preconditioning, a specific form of myocardial adaptive response, occurs through both G proteins and receptor tyrosine kinase. Such preconditioning, mediated by cyclic episodes of brief reversible ischemia each followed by another brief period of reperfusion, leads to improvement in infarct size and ventricular recovery. Adaptation can also be achieved through other environmental stresses, including oxidative stress. Several triggers for signal transduction have been identified, including catecholamines, bradykinin, and adenosine. The processing of stress signals from signal initiation to propagation to eventual termination is the focus of this chapter. Signal initiation occurs through G-protein as well as receptor tyrosine kinase activation. Complex signaling processes involving MAP kinases, MAPKAP kinase 2, and protein kinase C have become central to our understanding of signal propagation. Signal termination resulting in biol. expression of signal propagation by means of gene expression and transcription regulation is the ultimate outcome of cellular stress response. This chapter especially examines these complex signal transduction processes that lead to the stress response and eventual adaptation, focusing primarily upon protein kinase C-dependent and -independent pathways.

REFERENCE COUNT: 92 THERE ARE 92 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s parathyroid (w)hormone##  
 L4 119389 PARATHYROID (W) HORMONE##

=> sd his  
 SD IS NOT A RECOGNIZED COMMAND  
 The previous command name entered was not recognized by the system.  
 For a list of commands available to you in the current file, enter  
 "HELP COMMANDS" at an arrow prompt (=>).

=> d his

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
 L2 5 S L1 AND (MAPKAP-2 (W)KINASE?)  
 L3 4 DUP REM L2 (1 DUPLICATE REMOVED)  
 L4 119389 S PARATHYROID (W)HORMONE##

=> s l1 and l4  
 L5 6 L1 AND L4

=> dup rem l5  
 PROCESSING COMPLETED FOR L5  
 L6 5 DUP REM L5 (1 DUPLICATE REMOVED)

=> d 1-5 ibib ab

L6 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

DUPLICATE 1

ACCESSION NUMBER: 2005-12892 BIOTECHDS

TITLE: New gene expression profile of bone cells subjected to bone load modulated by a Wnt pathway modulator, useful for studying bone mineralization, identifying agents regulating bone mineralization and treating bone disorders;  
for bone disorder, osteoporosis, hypercalcemia, hyperostosis, osteoarthritis and osteomyelitis therapy

AUTHOR: CHATTERJEE-KISHORE M; ROBINSON J A; BHAT B M; BEX F J

PATENT ASSIGNEE: WYETH

PATENT INFO: WO 2005028678 31 Mar 2005

APPLICATION INFO: WO 2004-US17951 7 Jun 2004

PRIORITY INFO: US 2003-501398 10 Sep 2003; US 2003-476164 6 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-242579 [25]

AB DERWENT ABSTRACT:

NOVELTY - A gene expression profile of bone cells subjected to bone load that has been modulated by a Wnt pathway modulator, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) identifying Wnt pathway modulating agents and modulating bone remodeling, comprising: (a) obtaining a gene expression profile of bone cells exposed to a candidate agent; and (b) comparing the gene expression profile with the gene expression profile cited above to determine whether the Wnt pathway was modulated; (2) a gene expression profile of HBM cells subjected to mechanical stress and a Wnt pathway modulator; (3) preparing a bone loading gene expression profile, comprising: (a) obtaining a first gene expression profile of bone cells which are not exposed to bone load, a second gene expression profile of bone cells which are exposed to bone load, and a third gene expression profile of bone cells which are exposed to bone load and a Wnt pathway modulator, and (b) comparing the first, second and third gene expression profiles to obtain a bone loading gene expression profile of Wnt pathway modulator regulated genes; (4) a bone loading gene expression profile comprising genes regulated by a Wnt pathway modulator obtained by the method of (3); (5) screening an agent which enhances bone load associated remodeling, comprising: (a) obtaining a gene expression profile of bone cells cultured with the agent and exposed to bone load; and (b) comparing the gene expression profile with the bone loading gene expression profiles of (3), where the Wnt pathway modulator is a reference Wnt pathway modulator; (6) a candidate agent for treating a low bone mass condition identified by the method of (5); (7) treating a bone mineralization disease or disorder, comprising administering the candidate agent of (6); (8) a composition comprising probes having nucleic acid sequences that anneal to nucleic acids of the bone loading gene expression profile of (4); (9) modulating bone mineralization in a cell, comprising administering an agent which produces any of the bone load expression profiles cited; (10) modulating bone mineralization and/or bone remodeling in a subject, comprising administering a compound which produces a bone load expression profile of (4); (11) a composition comprising a substrate and a plurality of immunoglobulins adhered to the substrate, where the immunoglobulins recognize and bind to two or more of 274 proteins given in the specification as Tables 1-5, 11, or 12; (12) a composition for studying bone load modulation, comprising a substrate, and a plurality of two bone cell lysates or more cell lysates adhered to the substrate, where the lysate is from cells without mechanical stress, cells exposed to mechanical stress, HBM cells without mechanical stress, HBM cells exposed to mechanical stress, or any of the prior cells exposed to a Wnt pathway modulator; (13) screening reagents that bind to proteins that modulate bone remodeling and/or bone mineralization, comprising: (a) exposing a candidate reagent to a composition of (12) for binding of the candidate reagent to the composition; and (b) determining whether the candidate reagent bound to the composition and further determining which protein of the composition bound the candidate reagent; and (14)

determining whether a compound or a composition enhances the effect of bone load on bone cell activity/function and/or mineralization, comprising: (a) administering the compound or the composition to a cell line; (b) administering a mechanical stimulus to the cell line; (c) obtaining a cell lysate from the cell line; (d) contacting the cell lysate to the composition of (12) to allow binding of proteins in the cell lysate to the composition; and (e) determining whether the compound or the composition enhances the effect of bone load on bone cell activity/function and/or mineralization by comparing the pattern obtained with an expression pattern obtained from a cell lysate of cells to which mechanical load stimulus only was administered.

**BIOTECHNOLOGY - Preferred Expression Profile:** The gene expression profile comprises COX-2, Jun, Fos, SFRP1, Connexin 43, and eNOS genes, and two or more genes of Tables 1-5, 11, or 12. The Wnt pathway modulator is an agonist that is a GSK-3 inhibitor. The agonist is also a Wnt 3 A, a Wnt 3 A variant, a Wnt 3 A mimetic, or Wnt 3 A agonist. The GSK-3 inhibitor is a selective GSK-3 inhibitor, preferably a lithium chloride or its pharmaceutical salt, a maleimide, a muscarinic agonist, an aloisine, a hymeninidisine, or an inidirubin. The maleimide is 3-(2,4-dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione or 3-(3-chloro-4-hydroxyphenylamino)-4-(2-nitrophenyl)-1H-pyrrole-2,5-dione. The gene expression profile is derived from cultured cells or cells obtained from animal tissue. The bone cells are preosteoblasts, osteoprogenitor cells, osteoblasts, osteoclasts, osteocytes, or mesenchymal stem cells, or their combinations. **Preferred Method:** The mechanical load in identifying Wnt pathways is applied to an animal and the bone cells are obtained from the animal, where mechanical load is applied to cultured bone cells. The bone cells in preparing a bone loading gene expression profile are osteoclasts, osteoblasts, osteocytes, or a combination of the bone cells. The Wnt pathway modulator is a Wnt pathway agonist, preferably a GSK-3 inhibitor, a Wnt 3A, a Wnt 3A mimetic, a Wnt 3A agonist, a LRP5 agonist, a LRP6 agonist, a beta-catenin agonist, or a Dkk1 antagonist. The reference Wnt pathway modulator in screening an agent is a GSK-3 inhibitor or Wnt 3A. The cultured bone cell assessed in the absence of a candidate agent is an HBM bone cell. The bone cells are osteoblasts, preosteoblasts, osteoprogenitor cells, mesenchymal stem cells, or their combinations, preferably osteoblasts, and where the effect of the agent on osteoblast number and/or proliferation is measured by (3H)-thymidine incorporation, 5-bromo-2'-deoxyuridine (BrdU) incorporation, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium salt (MTS) assay, or an apoptosis assay. The bone load administered is mechanical load in the amount of 50-5000 micro-epsilon. The bone disease or disorder treated is osteoporosis, a bone fracture, chondrodystrophies, a drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteoarthritis, osteomyelitis, or Paget's disease. The bone fracture is a hip fracture, Colle's fracture, or a vertebral crush fracture. The drug-induced disorder is glucocorticoid induced osteoporosis, heparin-induced osteoporosis, an aluminum hydroxide induced osteomalacia, anticonvulsant induced osteomalacia, or glutethimide induced osteomalacia. The candidate agent is a GSK-3 antagonist, a Wnt 3A, a Wnt 3A mimetic, a Wnt 3A agonist, a Dkk1 antagonist, an LRP5 agonist, a beta-catenin agonist, or a LRP6 agonist. The agent in modulating bone mineralization is a Wnt agonist, a Wnt 3A, a Wnt 3A mimetic, a Wnt 3A variant, a Wnt 3A agonist, a Dkk antagonist, a COX-2 antagonist, a LRP5 agonist, a LRP6 agonist, a GSK-3 antagonist, or a beta-catenin agonist. The GSK-3 antagonist is a maleimide, a muscarinic agonist, an aloisine, a hymeninidisine or an inidirubin. The maleimide is administered in combination with a second bone remodeling modulating agent. The second bone remodeling modulating agent is parathyroid hormone, estrogen, vitamin D, a vitamin D analog, a selective estrogen receptor modulator, a glucocorticoid, a calcium preparation or a bisphosphonate. **Preferred Composition:** The probes in the composition of (8) are attached to a solid substrate that is a bead, a plate, or a



slide, and comprise nucleic acid sequences which anneal to nucleic acids sequences encoding connexin 43, COX-2, eNOS, SFRP1, Jun, and Fos proteins, and nucleic acid sequences that anneal to nucleic acid sequences of genes or gene transcripts of Tables 1-5, 11, or 12. The composition further comprises probes that anneal to nucleic acid sequences of PDGFRA, MET, OSMR, ITGBL1, CTGF, WNT6, TEV1P3, GJA1, GAS6, LOX, MYBL1, THBS1, ITGB5, CTSK, COL1A1, FBLN1, CCND1, TIMP2, COL6A3, GADD45A, WISP2, FZD2, SFRP4, IGFBP6, LRP5, LRP6, LSP1, CX3CR1, TRFBR2, VCAM1, IL6, FGF2, FGF7, STAT1, TNFRSF10B, IFG2R, IGF2, SPARC, MAPKAPK2, TNF, TNFRSF11b, TNFSF11, ACP5, FAP, MCC, DELTEX, EPHB2, CNK1, ERBB3, GRO1, MYC, or WNT10B. The immunoglobulins in the composition of (11) comprises two or more immunoglobulins that recognize and bind to said two or more proteins of Tables 1-5, 11, or 12. The two or more proteins are eNOS, connexin 43, SFRP1, cyclin D1, Wnt10B, Jun, Fos, or COX-2. The substrate is a microchip, a bead, a plate, a slide, or a tube. The substrate in the composition of (12) is a microchip, a bead, a plate, a slide or a tube.

ACTIVITY - Osteopathic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Wnt-Modulator.

USE - The methods and compositions of the present invention are useful for studying bone mineralization, identifying agents that regulate bone mineralization and for treating bone disorders, including osteoporosis, bone fracture, chondrodystrophies, a drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteoarthritis, osteomyelitis, or Paget's disease.

ADMINISTRATION - Dosage of the pharmaceutical composition ranges from 0.1-100, preferably 1-50 mg/kg. Routes of administration of the pharmaceutical compositions include oral, subcutaneous, rectal, inhalation, transdermal and parenteral routes. (174 pages)

L6 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:1314101 HCAPLUS

DOCUMENT NUMBER: 144:68263

TITLE: Genes showing altered levels of expression in drug-resistant leukemia and their use in diagnosis and selection of drug target for therapy

INVENTOR(S): Evans, William E.; Pieters, Rob; Cheok, Meyling H.; Den Boer, Monique L.; Yang, Wenjian

PATENT ASSIGNEE(S): St. Jude Children's Research Hospital, USA; Erasmus University Medical Center Rotterdam

SOURCE: PCT Int. Appl., 124 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005118865	A2	20051215	WO 2005-US17424	20050518
WO 2005118865	A3	20060622		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW			
RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.:

US 2004-575762P

P 20040528

AB The present invention encompasses methods and compns. useful in the diagnosis and treatment of drug resistant leukemia. The invention provides a number of genes that are differentially expressed between drug resistant and drug sensitive acute lymphoblastic leukemia (ALL). These genes act as biomarkers for drug resistant leukemia, and further serve as mol. targets for drugs useful in treating drug resistant leukemia. Accordingly, the invention provides methods of diagnosing drug resistant leukemia and methods of selecting a therapy for subjects affected by drug-resistant leukemia. The invention also provides methods for screening for compds. for treating drug-resistant leukemia, and improved methods for treating drug-resistant leukemia. Compns. of the invention include arrays, computer readable media, and kits for use in the methods of the invention.

L6 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2005:902703 HCAPLUS  
DOCUMENT NUMBER: 143:272498  
TITLE: Gene expression profiles in the diagnosis and treatment of Alzheimer's disease  
INVENTOR(S): Landfield, Philip W.; Porter, Nada M.; Chen, Kuey Chu; Geddes, James; Blalock, Eric  
PATENT ASSIGNEE(S): University of Kentucky Research Foundation, USA  
SOURCE: PCT Int. Appl., 114 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005076939	A2	20050825	WO 2005-US3668	20050209
WO 2005076939	A3	20060706		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, SM			
RW:	BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2004-542281P P 20040209

AB Genes showing altered patterns of expression in the brain that are associated with the neurol. changes found in Alzheimer's disease and that can be used in the early diagnosis of the disease, including the incipient form of the disease, are identified. The methods and kits of the invention utilize a set of genes and their encoded proteins that are shown to be correlated with incipient Alzheimer's disease.

L6 ANSWER 4 OF 5 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2005:1115611 SCISEARCH  
THE GENUINE ARTICLE: 980UU  
TITLE: ERK1/2-activated de novo Mapkapk2 synthesis is essential for osteogenic growth peptide mitogenic signaling in osteoblastic cells  
AUTHOR: San Miguel S M; Namdar-Attar M; Noh T; Frenkel B; Bab I (Reprint)  
CORPORATE SOURCE: Hebrew Univ Jerusalem, Bone Lab, POB 12272, IL-91120 Jerusalem, Israel (Reprint); Hebrew Univ Jerusalem, Bone Lab, IL-91120 Jerusalem, Israel; Univ So Calif, Keck Sch

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COUNTRY OF AUTHOR: Israel; USA  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (11 NOV 2005) Vol. 280,  
No. 45, pp. 37495-37502.  
ISSN: 0021-9258.  
PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650  
ROCKVILLE PIKE, BETHESDA, MD 20814-3996 USA.  
DOCUMENT TYPE: Article; Journal  
LANGUAGE: English  
REFERENCE COUNT: 51  
ENTRY DATE: Entered STN: 17 Nov 2005  
Last Updated on STN: 17 Nov 2005

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In osteoblasts, the mitogen-activated protein kinases ERK1/2 and p38 as well as the cAMP-response element-binding protein ( CREB) have been implicated in the regulation of proliferation and differentiation. The osteogenic growth peptide (OGP) is a 14-mer bone cell mitogen that increases bone formation and trabecular bone density and stimulates fracture healing. OGP-(10-14) is the physiologically active form of OGP. Using gene array analysis, real-time reverse transcription-PCR, and immunoblot and DNA synthesis assays we show here that in MC3T3E1 and newborn mouse calvarial osteoblastic cultures the OGP-( 10 - 14) mitogenic signaling is critically dependent on de novo synthesis of mitogen-activated protein kinase-activated protein kinase 2 ( Mapkapk2) mRNA and protein. The increase in Mapkapk2 occurs following short term ( 5 - 60 min) stimulation of ERK1/2 activity by OGP-( 10 - 14); phosphorylation of p38 remains unaffected. Downstream of Mapkapk2, CREB is phosphorylated on Ser133 leading to its enhanced transcriptional activity. That these events are critical for the OGP-( 10 - 14) mitogenic signaling is demonstrated by blocking the effects of OGP( 10 - 14) on the ERK1/2 pathway, Mapkapk2, CREB, and DNA synthesis using the MEK inhibitor PD098059. The OGP-( 10 - 14) stimulation of CREB transcriptional activity and DNA synthesis is also blocked by Mapkapk2 siRNA. These data define a novel mitogenic signaling pathway in osteoblasts whereby ERK1/2 stimulation of CREB phosphorylation and transcriptional activity as well as DNA synthesis are critically dependent on de novo Mapkapk2 synthesis.

L6 ANSWER 5 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-08555 BIOTECHDS

TITLE: New isolated nucleic acid molecule encoding a human  
mitogen-activated protein kinase activating protein kinase-2  
(MAPKAP-2), useful for treating immune-system  
related disorders, inflammation and arthritis;  
recombinant enzyme protein production and sense and  
antisense sequence for use in gene therapy

AUTHOR: LOGRASSO P; HAWKINS J; LISNOCK J M

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002090524 14 Nov 2002

APPLICATION INFO: WO 2002-US5670 25 Feb 2002

PRIORITY INFO: US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-111970 [10]

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences

that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if it is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to subtracting the product from  $X_a$ . The nucleic acid molecule has a sequence



that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the

sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay. In (M13), the allelic variant is encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound. (M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a

control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

ADMINISTRATION - Administration is oral, topical or parenteral (e.g. intraarterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal). The dosage is 0.001-50 mg/kg, preferably 0.1-1.0 mg/kg.

EXAMPLE - No relevant example given. (150 pages)

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
L2 5 S L1 AND (MAPKAP-2 (W)KINASE?)  
L3 4 DUP REM L2 (1 DUPLICATE REMOVED)  
L4 119389 S PARATHYROID (W)HORMONE##  
L5 6 S L1 AND L4  
L6 5 DUP REM L5 (1 DUPLICATE REMOVED)

=> s parathyroid (w)hormone-2

L7 221 PARATHYROID (W) HORMONE-2

=> s l1 and l7

L8 1 L1 AND L7

=> d all

L8 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
AN 2003-08555 BIOTECHDS  
TI New isolated nucleic acid molecule encoding a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2), useful for treating immune-system related disorders, inflammation and arthritis; recombinant enzyme protein production and sense and antisense sequence for use in gene therapy  
AU LOGRASSO P; HAWKINS J; LISNOCK J M  
PA MERCK and CO INC  
PI WO 2002090524 14 Nov 2002  
AI WO 2002-US5670 25 Feb 2002  
PRAI US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001  
DT Patent  
LA English  
OS WPI: 2003-111970 [10]  
AB DERWENT ABSTRACT:  
NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of



nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if it is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid

alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to subtracting the product from  $X_a$ . The nucleic acid molecule has a sequence that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter

gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay. In (M13), the allelic variant is encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound. (M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a



polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

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EXAMPLE - No relevant example given. (150 pages)

CC THERAPEUTICS, Protein Therapeutics; GENETIC TECHNIQUES and APPLICATIONS, Gene Expression Techniques and Analysis; GENETIC TECHNIQUES and APPLICATIONS, Genomic Technologies; DISEASE, Cancer; DISEASE, Other Diseases; DIAGNOSTICS, Molecular Diagnostics; THERAPEUTICS, Gene Therapy  
CT HUMAN RECOMBINANT MITOGEN-ACTIVATED PROTEIN-KINASE PREP., VECTOR-MEDIATED GENE TRANSFER EXPRESSION IN GENETICALLY ENGINEERED BACTERIUM, AMPHIBIAN OOCYTE HOST CELL, ANTIBODY, DNA PROBE, ANTAGONIST, AGONIST, SENSE, ANTISENSE SEQUENCE, RIBOZYME, TUMOR NECROSIS FACTOR, INTERLEUKIN-1, APPL. DRUG SCREENING, IMMUNE-RELATED DISORDER, CELL PROLIFERATIVE DISORDER, IMMUNE SYSTEM DISORDER, INFLAMMATION, ARTHRITIS THERAPY, DIAGNOSIS, CHROMOSOME MAPPING, TISSUE TYPING, PHARMACOGENOMICS, CLINICAL TRIAL, GENE THERAPY ANIMAL MAMMAL ENZYME EC-2.7.1.37 HYBRIDIZATION RNA ENZYME LYMPHOKINE ANTITUMOR CYTOKINE PROTEIN IMMUNOMODULATOR ANTIINFLAMMATORY CYTOSTATIC ANTIRHEUMATIC DNA SEQUENCE PROTEIN SEQUENCE (22, 14)

=> d kwic

L8 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
TI New isolated nucleic acid molecule encoding a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2), useful for treating immune-system related disorders, inflammation and arthritis; recombinant enzyme protein production and sense and antisense sequence for use.  
AB. . . isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, . . . fully complementary, or if its is RNA, is identical to mRNA native to

a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino. . . bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been. . . regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and. . . and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared. . . that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those. . . a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the. . . compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a

level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression. . . to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with. . . probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and. . . level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter. . . induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in. . . of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may. . . agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e)

determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY -. . . an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used. . .

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
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L4 119389 S PARATHYROID (W)HORMONE##  
L5 6 S L1 AND L4  
L6 5 DUP REM L5 (1 DUPLICATE REMOVED)  
L7 221 S PARATHYROID (W)HORMONE-2  
L8 1 S L1 AND L7

=> s immun? and l7

L9 68 IMMUN? AND L7

=> s l1 and l9

L10 1 L1 AND L9

=> d ibib ab

L10 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-08555 BIOTECHDS

TITLE: New isolated nucleic acid molecule encoding a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2), useful for treating immune -system related disorders, inflammation and arthritis; recombinant enzyme protein production and sense and antisense sequence for use in gene therapy

AUTHOR: LOGRASSO P; HAWKINS J; LISNOCK J M

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002090524 14 Nov 2002

APPLICATION INFO: WO 2002-US5670 25 Feb 2002

PRIORITY INFO: US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-111970 [10]

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.



DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if it is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to

subtracting the product from Xa. The nucleic acid molecule has a sequence that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying

ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay. In (M13), the allelic variant is encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound. (M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized

to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

ADMINISTRATION - Administration is oral, topical or parenteral (e.g. intraarterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal). The dosage is 0.001-50 mg/kg, preferably 0.1-1.0 mg/kg.

EXAMPLE - No relevant example given. (150 pages)

=> s skeletal (w)muscle

L11 369433 SKELETAL (W) MUSCLE

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
L2 5 S L1 AND (MAPKAP-2 (W)KINASE?)  
L3 4 DUP REM L2 (1 DUPLICATE REMOVED)  
L4 119389 S PARATHYROID (W)HORMONE##  
L5 6 S L1 AND L4  
L6 5 DUP REM L5 (1 DUPLICATE REMOVED)  
L7 221 S PARATHYROID (W)HORMONE-2  
L8 1 S L1 AND L7  
L9 68 S IMMUN? AND L7  
L10 1 S L1 AND L9  
L11 369433 S SKELETAL (W)MUSCLE

=> 's l4 and l11

'S IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (=>).

=> s l4 and l11

L12 387 L4 AND L11

=> s l7 and l11

L13 0 L7 AND L11

=> d l7 210-221

L7 ANSWER 210 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN



AN 1985:575780 HCAPLUS  
 DN 103:175780  
 TI Glycoproteins and phosphoproteins secreted by cultured chicken osteoblasts  
 AU Lowik, Clemens W. G. M.; Feyen, Jean H. M.; Van Zeeland, J. Karel;  
 Herrmann-Erlee, Maria P. M.  
 CS Lab. Cell Biol. Histol., Univ. Leiden, Leiden, 2333 AA, Neth.  
 SO International Congress Series (1985), 643(Curr. Adv. Skeletogenesis),  
 99-104  
 CODEN: EXMDA4; ISSN: 0531-5131  
 DT Journal  
 LA English

L7 ANSWER 211 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1984:527910 HCAPLUS  
 DN 101:127910  
 TI Fetal bovine bone cells synthesize bone-specific matrix proteins  
 AU Whitson, S. William; Harrison, Wilbur; Dunlap, Mary K.; Bowers, Daniel E.,  
 Jr.; Fisher, Larry W.; Robey, Pamela Gehron; Termine, John D.  
 CS Sch. Dent. Med., South. Illinois Univ., Alton, IL, 62002, USA  
 SO Journal of Cell Biology (1984), 99(2), 607-14  
 CODEN: JCLBA3; ISSN: 0021-9525  
 DT Journal  
 LA English

L7 ANSWER 212 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1980:213199 HCAPLUS  
 DN 92:213199  
 TI Parathyroid hormone metabolism and electrolyte excretion in healthy  
 controls and patients with nephrolithiasis  
 AU Dunzendorfer, U.; Schmidt-Gayk, H.  
 CS Abt. Urol., Universitaetsklin. Frankfurt, Frankfurt/Main, Fed. Rep. Ger.  
 SO Klinische Wochenschrift (1980), 58(3), 153-5  
 CODEN: KLWOAZ; ISSN: 0023-2173  
 DT Journal  
 LA German

L7 ANSWER 213 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1979:518888 HCAPLUS  
 DN 91:118888  
 TI Identification and cell-free translation of mRNA coding for a precursor of  
 parathyroid secretory protein  
 AU Majzoub, Joseph A.; Kronenberg, Henry M.; Potts, John T., Jr.; Rich,  
 Alexander; Habener, Joel F.  
 CS Howard Hughes Med. Inst., Harvard Med. Sch., Boston, MA, 02114, USA  
 SO Journal of Biological Chemistry (1979), 254(15), 7449-55  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English

L7 ANSWER 214 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1977:165612 HCAPLUS  
 DN 86:165612  
 TI States of activation of chick kidney adenylate cyclase induced by  
 parathyroid hormone and guanyl nucleotides  
 AU Michelangeli, V. P.; Hunt, N. H.; Martin, T. J.  
 CS Med. Sch., Sheffield Univ., Sheffield, UK  
 SO Journal of Endocrinology (1977), 72(1), 69-79  
 CODEN: JOENAK; ISSN: 0022-0795  
 DT Journal  
 LA English

L7 ANSWER 215 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1975:558083 HCAPLUS  
 DN 83:158083

TI Parathyroid hormone. 2. Physiology.  
 Hormonal effects  
 AU Copp, D. Harold  
 CS Dep. Physiol., Univ. British Columbia, Vancouver, BC, Can.  
 SO Methods in Investigative and Diagnostic Endocrinology (1973), 2B(Pept.  
 Horm.), 955-60  
 CODEN: MIDECF  
 DT Journal; General Review  
 LA English

L7 ANSWER 216 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1972:509625 HCAPLUS  
 DN 77:109625  
 TI Bone resorption in vitro  
 AU Reynolds, John J.  
 CS Strangeways Res. Lab., Cambridge, UK  
 SO Quarterly Journal of Surgical Sciences (1970), 6(2-3), 63-7  
 CODEN: QJSSAB; ISSN: 0033-5657  
 DT Journal  
 LA English

L7 ANSWER 217 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
 AN 1968:441480 HCAPLUS  
 DN 69:41480  
 TI Time course of action of calcitonin on resorbing mouse bones in vitro  
 AU Reynolds, J. J.; Dingle, J. T.  
 CS Strangeways Res. Lab., Cambridge, UK  
 SO Nature (London, United Kingdom) (1968), 218(5147), 1178-9  
 CODEN: NATUAS; ISSN: 0028-0836  
 DT Journal  
 LA English

L7 ANSWER 218 OF 221 LIFESCI COPYRIGHT 2006 CSA on STN  
 AN 2003:86704 LIFESCI  
 TI Tuberoinfundibular peptide of 39 residues (TIP39): molecular structure and  
 activity for parathyroid hormone 2 receptor  
 AU Penna, K.D.; Kinose, F.; Sun, H.; Koblan, K.S.; Wang, H.\*  
 CS Department of Molecular Pharmacology, Merck Research Laboratories, West  
 Point, PA 19486, USA  
 SO Neuropharmacology, (20030100) vol. 44, no. 1, pp. 141-153.  
 ISSN: 0028-3908.  
 DT Journal  
 FS N3  
 LA English  
 SL English

L7 ANSWER 219 OF 221 LIFESCI COPYRIGHT 2006 CSA on STN  
 AN 2003:77906 LIFESCI  
 TI Expression and Distribution of Tuberoinfundibular Peptide of 39 Residues  
 in the Rat Central Nervous System  
 AU Dobolyi, A.; Palkovits, M.; Usdin, T.B.  
 CS Laboratory of Genetics, National Institute of Mental Health, 36 Convent  
 Dr., Bethesda, MD 20892-4094, USA; E-mail: usdin@codon.nih.gov  
 SO Journal of Comparative Neurology [J. Comp. Neurol.], (20021209) vol. 455,  
 no. 4, pp. 547-566.  
 ISSN: 0021-9967.  
 DT Journal  
 FS N3  
 LA English  
 SL English

L7 ANSWER 220 OF 221 LIFESCI COPYRIGHT 2006 CSA on STN  
 AN 2002:81688 LIFESCI  
 TI Anatomical and physiological evidence for involvement of

tuberoinfundibular peptide of 39 residues in nociception  
AU Dobolyi, A.; Ueda, H.; Uchida, H.; Palkovits, M.; Usdin, T.B.  
CS Laboratory of Genetics, National Institute of Mental Health, Bethesda, MD  
20892, USA; E-mail: usdin@codon.nih.gov  
SO Proceedings of the National Academy of Sciences, USA [Proc. Natl. Acad.  
Sci. USA], (20020205) vol. 99, no. 3, pp. 1651-1656.  
ISSN: 0027-8424.  
DT Journal  
FS N3  
LA English  
SL English

L7 ANSWER 221 OF 221 LIFESCI COPYRIGHT 2006 CSA on STN  
AN 94:94218 LIFESCI  
TI Characterization of the mineralization process in cultures of rabbit  
growth plate chondrocytes  
AU Jikko, A.; Aoba, T.; Murakami, H.; Takano, Y.; Iwamoto, M.; Kato, Y.\*  
CS Dep. Biochem., Sch. Dent., Hiroshima Univ., Hiroshima, Japan  
SO DEV. BIOL., (1993) vol. 156, no. 2, pp. 372-380.  
ISSN: 0012-1606.  
DT Journal  
FS T  
LA English  
SL English

=> d 17 218 ibib ab

L7 ANSWER 218 OF 221 LIFESCI COPYRIGHT 2006 CSA on STN  
ACCESSION NUMBER: 2003:86704 LIFESCI  
TITLE: Tuberoinfundibular peptide of 39 residues (TIP39):  
molecular structure and activity for parathyroid  
hormone 2 receptor  
AUTHOR: Penna, K.D.; Kinose, F.; Sun, H.; Koblan, K.S.; Wang, H.\*  
CORPORATE SOURCE: Department of Molecular Pharmacology, Merck Research  
Laboratories, West Point, PA 19486, USA  
SOURCE: Neuropharmacology, (20030100) vol. 44, no. 1, pp. 141-153.  
ISSN: 0028-3908.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: N3  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB The neuropeptide TIP39 was recently purified from bovine hypothalamus  
based on the ability of the peptide to activate the parathyroid  
hormone 2 receptor (PTH2R) (Usdin et al. Nat. Neurosci.  
2 (1999) 941). PTH2R is abundantly expressed in the nervous system, and  
its expression pattern suggests that it may play a role in modulation of  
pituitary function and in nociception. Towards understanding the  
physiological role of TIP39 and PTH2R, we cloned human, mouse and rat  
TIP39 gene. Our results revealed that: (1) the mature peptide is processed  
from a precursor; (2) TIP39 peptide is highly conserved among species; and  
(3) TIP39 from all species activates adenylyl cyclase and elevates  
intracellular calcium levels through PTH2R. We also defined and compared  
the structure-activity relationship of TIP39 on both activation of  
adenylyl cyclase and calcium mobilization pathways through PTH2R, finding  
common and differential determinants of TIP39 that are required for these  
pathways. Furthermore, we observed that TIP39 elevates intracellular  
calcium levels in primary dorsal root ganglion neurons whereas the peptide  
inactive on PTH2R do not, suggesting that TIP39 may activate these neurons  
important for nociception in vivo through PTH2R-dependent mechanisms.

=> d 17 200-210 ibib ab



L7 ANSWER 200 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1996:580502 HCAPLUS

DOCUMENT NUMBER: 125:293602

TITLE: Distribution of parathyroid hormone  
-2 receptor messenger ribonucleic acid in  
rat

AUTHOR(S): Usdin, Ted B.; Bonner, Tom I.; Harta, Gyongyi; Mezey,  
Eva

CORPORATE SOURCE: Lab. Cell Biol., Natl. Inst. Health, Bethesda, MD,  
20892, USA

SOURCE: Endocrinology (1996), 137(10), 4285-4297

CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We obtained a cDNA encoding the rat PTH2 receptor and used it to study the distribution of the PTH2 receptor using in situ hybridization histochem. PTH2 receptor mRNA is abundantly expressed in arterial and cardiac endothelium and at lower levels in vascular smooth muscle. It is also abundant in the lung, both within bronchi and in the parenchyma, and is present within the exocrine pancreas. It is expressed by sperm in the head of the epididymis. A small number of cells associated with the vascular pole of renal glomeruli express the receptor. These data suggest that the PTH2 receptor may be responsible for PTH effects in a number of physiol. systems.

L7 ANSWER 201 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1995:460422 HCAPLUS

DOCUMENT NUMBER: 122:205722

TITLE: Acute effect of parathyroid hormone on urine  
concentration in the rat

AUTHOR(S): Carney, S. L.; Gillies, A. H. B.

CORPORATE SOURCE: Faculty Medicine, University Newcastle, Newcastle,  
Australia

SOURCE: Clinical Science (1995), 88(2), 197-201

CODEN: CSCIAE; ISSN: 0143-5221

DOCUMENT TYPE: Journal

LANGUAGE: English

AB It has been demonstrated that parathyroid hormone can increase adenylate cyclase activity in the rat papilla, produce a small antidiuretic effect and in vitro can interfere with the action of arginine vasopressin on water transport. Clearance studies were performed in the anesthetized water diuretic thyroparathyroidectomized rat to evaluate further the effect of parathyroid hormone on urine concentration in the presence and absence

of arginine vasopressin. A maximal phosphaturic concentration of rat parathyroid hormone (2 µg/kg) reduced urine flow from 125 to 81 µL/min within 10 min. Addition of a maximal antidiuretic concentration of arginine vasopressin (100ng/kg) produced a delayed

and diminished antidiuretic response when compared with a group of rats not pretreated with parathyroid hormone (47 compared with 27 µL/min).

However, a supramaximal arginine vasopressin concentration (1000ng/kg) produced a

maximal antidiuretic effect in the presence of parathyroid hormone. To evaluate further the inhibitory effect of parathyroid hormone on arginine vasopressin-induced antidiuresis, parathyroid hormone (2µg/kg) was administered to one group of rats and a minimally effective arginine vasopressin concentration (7.5ng/kg) to another group, which produced a similar antidiuretic effect. However, the subsequent effect of a maximal antidiuretic arginine vasopressin concentration (100ng/kg) was again significantly blunted in the group pretreated with parathyroid hormone. Parathyroid hormone produced only a small increase in mean plasma calcium concentration, and glomerular filtration rate was not

altered by either hormone. These results demonstrate that high physiol. concns. of parathyroid hormone do have a significant antidiuretic effect and can interfere with the action of arginine vasopressin. This suggests that parathyroid hormone may act as a partial agonist to arginine vasopressin in the collecting system.

L7 ANSWER 202 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1993:219884 HCAPLUS  
DOCUMENT NUMBER: 118:219884  
TITLE: Pharmaceutical emulsion containing parathyroid hormone for pernasal administration  
INVENTOR(S): Yamamoto, Nakayuki; Sugimoto, Michihiko; Morimoto, Seiki; Sakakibara, Hideo; Saita, Masaru; Shimoazono, Yuji; Manako, Takafumi  
PATENT ASSIGNEE(S): Asahi Kasei Kogyo K. K., Japan; Hisamitsu Seiyaku K. K.  
SOURCE: PCT Int. Appl., 26 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9305805	A1	19930401	WO 1992-JP1179	19920916
W: AU, CA, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
JP 05070367	A2	19930323	JP 1991-236193	19910917
JP 3090353	B2	20000918		
AU 9225843	A1	19930427	AU 1992-25843	19920916
AU 662168	B2	19950824		
EP 610502	A1	19940817	EP 1992-919966	19920916
EP 610502	B1	19980722		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
CA 2118655	C	19970819	CA 1992-2118655	19920916
AT 168562	E	19980815	AT 1992-919966	19920916
KR 129865	B1	19980409	KR 1994-700867	19940317
US 5407911	A	19950418	US 1994-211035	19940516
PRIORITY APPLN. INFO.:			JP 1991-236193	A 19910917
			WO 1992-JP1179	A 19920916
OTHER SOURCE(S):	MARPAT 118:219884			
AB	A stable and readily absorbable intranasal emulsion contains (1) a parathyroid hormone, (2) an absorption enhancer, azacycloalkanes (I; R= alkyl; m= 2-4; n= 1-15; but when n= 1-3, R= C5-11 alkyl), (3) glycyrrhizinic acid or its salt, and (4) water. An intranasal formulation contained human parathyroid hormone 200 units, 1-[2-(decylthio)ethyl]azacyclopentan-2-one 5, 2K glycyrrhizinate 10, glycerin 22, methylparaben 1.2, propylparaben 0.3 mg, and water to 1.0 mL.			

L7 ANSWER 203 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 1993:188639 HCAPLUS  
DOCUMENT NUMBER: 118:188639  
TITLE: Characterization of the mineralization process in cultures of rabbit growth plate chondrocytes  
AUTHOR(S): Jikko, Akitoshi; Aoba, Takaaki; Murakami, Hiroshi; Takano, Yoshiro; Iwamoto, Masahiro; Kato, Yukio  
CORPORATE SOURCE: Fac. Dent., Osaka Univ., Suita, Japan  
SOURCE: Developmental Biology (Orlando, FL, United States) (1993), 156(2), 372-80  
CODEN: DEBIAO; ISSN: 0012-1606  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Chondrocytes were isolated from the growth plate of ribs of 4-wk-old

rabbits. The nature and properties of mineral crystals precipitated in chondrocyte cultures were compared with those of crystals formed in the hypertrophic zone and bone of rabbit rib growth plates in vivo. The chondrocytes were maintained at high d. on type II collagen-coated dishes in Eagle's medium,  $\alpha$ -modification, with 10% fetal bovine serum and 50  $\mu\text{g/mL}$  of ascorbic acid. These cells differentiated into hypertrophic cells 10 days after seeding and produced alkaline phosphatase and 1,25-dihydroxyvitamin D3 receptors on Days 30-70 at levels as high as those in the lower hypertrophic zone in vivo. Mineralization was initiated between Days 20 and 30 and advanced progressively throughout the culture period. However, mineralization was suppressed by the addition of parathyroid hormone ( $2 + 10^{-8}\text{M}$ ) or by the presence of fibroblasts. Examination by electron microscopy and Fourier transform IR (FTIR) spectroscopy verified that mineralized nodules formed in vitro were composed of small apatite crystals. Importantly, FTIR spectral features of the apatite crystals (e.g., the prominent  $\text{PO}_4$  bands at 1125 and 1032  $\text{cm}^{-1}$ ) were similar to those of cartilage apatites formed in vivo and differed markedly from those of carbonated bone apatites. These results suggest that growth plate chondrocytes cultured on collagen-coated dishes are an appropriate model for studies on cartilage mineralization.

L7 ANSWER 204 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1991:485575 HCAPLUS

DOCUMENT NUMBER: 115:85575

TITLE: Dissociation of second messenger activation by parathyroid hormone fragments in osteosarcoma cells  
AUTHOR(S): Fujimori, Akira; Cheng, Su Li; Avioli, Louis V.; Civitelli, Roberto

CORPORATE SOURCE: Div. Bone Miner. Metab., Jew. Hosp. St. Louis, St. Louis, MO, 63110, USA

SOURCE: Endocrinology (1991), 128(6), 3032-9  
CODEN: ENDOAO; ISSN: 0013-7227

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Structure-function relation of parathyroid hormone (PTH) was studied by analyzing the effects of bovine PTH-(1-34) [bPTH-(1-34)] and PTH fragments truncated at the N-terminus on the intracellular  $\text{Ca}$  concentration ( $[\text{Ca}^{2+}]_i$ ) and cAMP production in the rat osteogenic sarcoma cell line UMR 106-01.  $[\text{Ca}^{2+}]_i$  was measured in single cells using fura-2. When exposed to  $10^{-7}\text{M}$  bPTH-(1-34), 20% of the cells responded with a transient increase in  $[\text{Ca}^{2+}]_i$  of variable amplitude. Equimolar doses of bPTH-(2-34), propionyl bPTH-(2-34) [pbPTH-(2-34)], and bPTH-(3-34) also transiently increased  $[\text{Ca}^{2+}]_i$ , whereas both [tyrosine<sup>34</sup>]bPTH-(7-34) amide [bPTH-(7-34)] and bPTH-(30-34) were ineffective. The amplitude of the  $[\text{Ca}^{2+}]_i$  transients was dose-dependent, with threshold concns. of  $10^{-10}\text{M}$  for bPTH-(1-34) and bPTH-(2-34), and  $10^{-9}\text{M}$  for bPTH-(3-34). The response rate to the active peptides ranged 10-30%, without a clear dose-relatedness. A 2nd addition of  $10^{-7}\text{M}$  bPTH-(1-34) to cells prestimulated with equimolar doses of bPTH-(2-34), pbPTH-(2-34), or bPTH-(3-34) produced another transient, whereas after exposure to  $10^{-7}\text{M}$  bPTH-(1,34), the cells were completely desensitized to a 2nd homologous stimulation, suggesting that the binding affinity of the truncated peptides for the PTH receptor is lower than that of the intact bPTH-(1-34) fragment. In addition, both bPTH-(1-34) dose-dependently stimulated cAMP production, but the former was more potent ( $\text{ED}_{50} = 10^{-9}$  vs.  $10^{-7}\text{M}$ , resp.). On the contrary, pbPTH-(2-34), bPTH-(3-34), and bPTH-(7-34) had no effect on cAMP. Pretreating the cells with pertussis toxin to enhance cAMP responses via inhibition of  $\text{G}_i$  potentiated the effect of bPTH-(1-34) and bPTH-(2-34) and disclosed weak but detectable agonist action of pbPTH-(2-34). These results indicate that specific domains of the PTH mol. are linked to activation of different 2nd messenger pathways; while the 1st 2 amino acids are indispensable for activating the cAMP system, generation of the  $[\text{Ca}^{2+}]_i$  signal appears to involve a longer domain, including the amino acid

residue in position 3.

L7 ANSWER 205 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1991:221499 HCAPLUS

DOCUMENT NUMBER: 114:221499

TITLE: Intact parathyroid hormone assay is superior to mid region assay in the EDTA-infusion test in hyperparathyroidism

AUTHOR(S): Bergenfelz, Anders; Norden, Nils E.; Ahren, Bo

CORPORATE SOURCE: Dep. Surg., Lund Univ., Lund, S-221 85, Swed.

SOURCE: Clinica Chimica Acta (1991), 197(3), 229-35

CODEN: CCATAR; ISSN: 0009-8981

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An intact parathyroid hormone 2-site immunoradiometric assay was compared with a mid region parathyroid hormone RIA in the EDTA-infusion test in 15 patients with hyperparathyroidism. During the test, plasma intact parathyroid hormone levels increased by 240%, whereas the plasma levels of mid mol. parathyroid hormone increased by only 65%. Four patients had no increase in plasma mid mol. parathyroid hormone level but still a large increase in plasma intact parathyroid hormone level. Thus, plasma measurement of intact parathyroid hormone is superior to that of mid mol. parathyroid hormone in the EDTA-infusion test in patients with hyperparathyroidism.

L7 ANSWER 206 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1990:585135 HCAPLUS

DOCUMENT NUMBER: 113:185135

TITLE: The effect of parathyroid hormone on hemodynamic response to angiotensin-2 in normotensive and spontaneously hypertensive rats

AUTHOR(S): Brin, V. B.; Tatrov, A. S.

CORPORATE SOURCE: North-Ossetian Med. Inst., Ordzhonikidze, USSR

SOURCE: Fiziologicheskii Zhurnal SSSR imeni I. M. Sechenova (1990), 76(3), 345-50

CODEN: FZLZAM; ISSN: 0015-329X

DOCUMENT TYPE: Journal

LANGUAGE: Russian

AB The effect of parathyroid hormone (2 units/100 g) on the hemodynamic response to various doses of i.v. angiotensin II (16, 20 or 24 ng/100 g) was studied in normotensive and spontaneously hypertensive rats. The effect involved a decrease of the minute blood volume, stroke volume, and an increase of total peripheral resistance in normotensive rats. Parathyroid hormone decreased total peripheral resistance and increased the minute blood volume and the heart rate in spontaneously hypertensive rats. Angiotensin II raised the arterial pressure in normotensive rats. In the presence of parathyroid hormone, the angiotensin II pressor effect was quite obvious in normotensive rats, whereas in spontaneously hypertensive rats angiotensin II caused the greatest rise of arterial pressure as a result of a considerable dose-dependent rise of total peripheral resistance, the cardiac output showing a tendency towards a reduction

L7 ANSWER 207 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1989:33825 HCAPLUS

DOCUMENT NUMBER: 110:33825

TITLE: Clinical and laboratory evaluation of a two-site immunoradiometric assay for intact parathyroid hormone

AUTHOR(S): Newman, D. J.; Ashby, J. P.

CORPORATE SOURCE: Dep. Clin. Chem., Northwick Park Hosp.,

Harrow/Middlesex, HA1 3UJ, UK

SOURCE: Annals of Clinical Biochemistry (1988), 25(6), 654-60

CODEN: ACBOBU; ISSN: 0004-5632

DOCUMENT TYPE: Journal



LANGUAGE: English

AB The anal. performance and clin. utility of a direct immunoradiometric assay (IRMA) for intact 1-84 human parathyroid hormone (PTH) was evaluated. The assay is available com. and utilizes 2 affinity purified region-specific antisera against either 1-34 (radiolabeled antibody) or 39-84 (solid phase antibody) human PTH. High assay sensitivity (detection limit, 1.4 pg 1-84 PTH/mL) permitted the measurement of PTH in all normocalcemic individuals studied. Elevated results were obtained in all patients with histol. proven primary hyperparathyroidism (PHPT) and 34 out of 35 patients with presumptive PHPT. Thirteen out of 24 patients with non-parathyroid hypercalcemia had suppressed results, but the remainder had concns. within the reference range.

L7 ANSWER 208 OF 221. HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1988:486687 HCAPLUS

DOCUMENT NUMBER: 109:86687

TITLE: Induction of ornithine decarboxylase activity in isolated chicken osteoblasts by parathyroid hormone: the role of cAMP and calcium

AUTHOR(S): Lowik, C. W. G. M.; Olthof, A. A.; Van Leeuwen, J. P. T. M.; Van Zeeland, J. K.; Herrmann-Erlee, M. P. M.

CORPORATE SOURCE: Lab. Cell Biol. Histol., Univ. Leiden, Leiden, Neth.

SOURCE: Calcified Tissue International (1988), 43(1), 7-18

CODEN: CTINDZ; ISSN: 0171-967X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The roles of cAMP and Ca<sup>2+</sup> as mediators in parathyroid hormone (PTH)-induced ornithine decarboxylase (ODC) activity in primary cultures of chicken osteoblasts were examined. The induction of ODC activity by PTH is most likely a receptor-mediated process and cAMP is a mediator. However, 3 different approaches indicate that cAMP is not the exclusive mediator of PTH-induced ODC activity. First, when the dose-response curve of PTH-induced ODC activity is compared with that of PTH-stimulated cAMP production, the ED<sub>50</sub> for cAMP production is about 5 times as high as that for the induction of ODC activity. Second, 1 mM 9-(tetrahydro-2-furanyl)adenine (SQ 22.536) almost completely inhibited PTH-stimulated cAMP production whereas there was only a small inhibitory effect on PTH-induced ODC activity. Third, some PTH fragments unable to stimulate cAMP production were still able to induce ODC activity. Apart from cAMP, an addnl. messenger, most likely Ca<sup>2+</sup>, must be present. Evidence for this concept are the observations that substances affecting extracellular and intracellular Ca<sup>2+</sup> levels (EGTA, A 23187, CoCl<sub>2</sub>, verapamil) or antagonizing calmodulin (trifluoroperazin, compound 48/80) also strongly affect PTH-induced ODC activity. These effects could not be explained by a pos. interaction of Ca<sup>2+</sup> with the hormone-stimulated cAMP system as 2 mM EGTA strongly enhanced PTH-stimulated cAMP production but at the same time completely inhibited PTH-induced ODC activity. A similar dissociation between hormone-induced cAMP production and induction of ODC activity was found with the Ca<sup>2+</sup>-ionophore A 23187 (10<sup>-7</sup>M) which inhibited PTH-stimulated cAMP production but strongly enhanced PTH-induced ODC activity. Intracellular Ca<sup>2+</sup>, and possibly calmodulin, in addition to cAMP, are probably involved in PTH-induced ODC activity in chicken osteoblasts. Most probably Ca<sup>2+</sup> is the initial messenger and cAMP acts in a coordinate pattern as a synarchic messenger making the induction of ODC activity by PTH more sensitive to Ca<sup>2+</sup>. Furthermore, the present findings are in agreement with the concept of the existence of 2 receptors or 2 receptor-sites for PTH on osteoblasts. One receptor is coupled to the production of cAMP and is presumably activated when the 1st 2 amino acids of the NH<sub>2</sub>-terminus of the hormone are present and the other, suggested to be responsible for the increase in intracellular Ca<sup>2+</sup>, is thought to be activated by a region of the hormone sequence between amino acid 3 and 34. Activation of both receptors by the intact hormone PTH(1-84) or its biol. active fragment PTH(1-34) leads to the maximal induction of ODC activity.

L7 ANSWER 209 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1985:590337 HCAPLUS

DOCUMENT NUMBER: 103:190337

TITLE: Identification of a monoclonal antibody which interacts with the parathyroid hormone receptor-adenylate cyclase system in murine bone

AUTHOR(S): Weinshank, Richard L.; Cain, Christopher D.; Vasquez, Nora P.; Luben, Richard A.

CORPORATE SOURCE: Dep. Biochem., Univ. California, Riverside, CA, 92521, USA

SOURCE: Molecular and Cellular Endocrinology (1985), 41(2-3), 237-46

CODEN: MCEND6; ISSN: 0303-7207

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Monoclonal antibodies which bind specifically to mouse bone cells were produced and then selected for their ability to inhibit parathyroid hormone (PTH) responses in mouse cranial bone treated with the (1-34) bovine PTH [bPTH(1-34)] [12583-68-5]. One clone, designated 3-6, characterized as an IgM( $\kappa$ ), significantly inhibited the accumulation of cAMP [60-92-4] in response to bPTH(1-34) at concns. of  $10^{-9}$ - $10^{-7}$ M. This antibody was subsequently isolated by gel filtration and shown to bind to intact mouse calvariae, with saturation binding occurring at 3  $\mu$ g IgM/mL. A maximal inhibition of .apprx.70% of the cAMP accumulation produced in response to  $2.5 \times 10^{-8}$ M (100 ng/mL) bPTH(1-34) was obtained with 7  $\mu$ g of the purified 3-6 IgM/mL. At this concentration of 3-6 IgM, the half-maximal dose of PTH for activation of cAMP accumulation was increased from  $5 \times 10^{-9}$ M to  $2 \times 10^{-8}$ M with no reduction in maximal levels of cAMP production. The utility of this antibody as an inhibitor was further tested by its ability to block the binding of an iodinated PTH analog  $^{125}$ I-labeled [Nle<sup>8</sup>,Nle<sup>18</sup>,Tyr<sup>34</sup>]-bPTH(1-34) [59029-34-4] to mouse cranial bone. The 3-6 IgM at a concentration of  $5 \times 10^{-8}$ M inhibited 70% of the specific binding of the  $^{125}$ I-labeled analog. In the absence of parathyroid hormone,  $2 \times 10^{-8}$ M 3-6 IgM produced a 4-fold increase in cAMP above basal levels, as compared to 40-fold maximal increases observed with PTH, indicating a partial PTH agonist activity of this antibody. When tested for effects on other hormones, 3-6 IgM did not inhibit cAMP accumulation produced in response to salmon calcitonin, epinephrine, PGE<sub>2</sub>, or cholera toxin. Apparently the 3-6 monoclonal IgM is specific for the PTH receptor or a component of the PTH receptor-adenylate cyclase [9012-42-4] system and this or similar antibodies will serve as useful reagents for future mol. characterization of this receptor.

L7 ANSWER 210 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1985:575780 HCAPLUS

DOCUMENT NUMBER: 103:175780

TITLE: Glycoproteins and phosphoproteins secreted by cultured chicken osteoblasts

AUTHOR(S): Lowik, Clemens W. G. M.; Feyen, Jean H. M.; Van Zeeland, J. Karel; Herrmann-Erlee, Maria P. M.

CORPORATE SOURCE: Lab. Cell Biol. Histol., Univ. Leiden, Leiden, 2333 AA, Neth.

SOURCE: International Congress Series (1985), 643(Curr. Adv. Skeletogenesis), 99-104

CODEN: EXMDA4; ISSN: 0531-5131

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Osteoblasts, isolated from 18-day-old chick embryos, contd. 4 proteins of relative mol. wts. 40, 67, 170, and >200 kilodaltons (kD) which bound to a Con A affinity column. Pulse incubation of the osteoblast cells with  $^3$ H-labeled glucosamine for 8 h showed label uptake by the 170 and >200 kD proteins. Autoradiographs of osteoblasts incubated with  $^{32}$ P revealed the

presence of 15 phosphoproteins with a major product of 48 kD being formed after 16 h exposure. In the presence of parathyroid hormone (2 units/mL), 4 of the phosphoproteins formed after incubation with <sup>32</sup>P were not labeled and phosphorylation of a 35 kD protein was stimulated. Thus, chicken osteoblasts are able to synthesize and secrete glycoproteins and phosphoproteins and parathyroid hormone has a regulatory role in these processes.

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L7 ANSWER 190 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2000:691668 HCAPLUS  
DOCUMENT NUMBER: 134:80844  
TITLE: New Members of the Parathyroid Hormone/Parathyroid  
Hormone Receptor Family: The Parathyroid  
Hormone 2 Receptor and  
Tuberoinfundibular Peptide of 39 Residues  
AUTHOR(S): Usdin, Ted B.; Wang, Tianlun; Hoare, Samuel R. J.;  
Mezey, Eva; Palkovits, Miklos  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental  
Health, Bethesda, MD, 20892-4094, USA  
SOURCE: Frontiers in Neuroendocrinology (2000), 21(4), 349-383  
CODEN: FNEDA7; ISSN: 0091-3022  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal; General Review  
LANGUAGE: English  
AB A review, with 160 refs. The parathyroid hormone (PTH) family currently  
includes three peptides and three receptors. PTH regulates calcium  
homeostasis through bone and kidney PTH1 receptors. PTH-related peptide,  
probably also through PTH1 receptors, regulates skeletal, pancreatic,  
epidermal, and mammary gland differentiation and bladder and vascular  
smooth muscle relaxation and has a CNS role that is under investigation.  
Tuberoinfundibular peptide of 39 residues (TIP39) was recently purified  
from bovine hypothalamus based on selective PTH2 receptor activation.  
PTH2 receptor expression is greatest in the CNS, where it is concentrated in  
limbic, hypothalamic, and sensory areas, especially hypothalamic  
periventricular  
neurons, nerve terminals in the median eminence, superficial layers of the  
spinal cord dorsal horn, and the caudal part of the sensory trigeminal  
nucleus. It is also present in a number of endocrine cells. Thus TIP39 and  
PTH2 receptor-influenced functions may range from pituitary and pancreatic  
hormone release to pain perception. A third PTH-recognizing receptor has  
been found in zebrafish. (c) 2000 Academic Press.  
REFERENCE COUNT: 160 THERE ARE 160 CITED REFERENCES AVAILABLE FOR  
THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L7 ANSWER 191 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN  
ACCESSION NUMBER: 2000:641938 HCAPLUS  
DOCUMENT NUMBER: 133:276496  
TITLE: Structure of tuberoinfundibular peptide of 39 residues  
AUTHOR(S): Piserchio, Andrea; Usdin, Ted; Mierke, Dale F.  
CORPORATE SOURCE: Department of Chemistry, Brown University, Providence,  
RI, 02912, USA  
SOURCE: Journal of Biological Chemistry (2000), 275(35),  
27284-27290  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB The recently identified natural peptide ligand, tuberoinfundibular peptide  
of 39 residues (TIP39) for the parathyroid hormone-

2 (PTH2) receptor has been structurally characterized by high resolution NMR, CD, and computer simulations. The structural features of TIP39, determined in the presence of a zwitterionic lipid to mimic the membrane environment of the G-protein-coupled PTH2 receptor, consist of two  $\alpha$ -helices, Ala5-Arg21 and Leu26-Val35. Although TIP39 shares limited sequence homol. with parathyroid hormone (PTH), a comparison of the structural features of TIP39 and PTH illustrates a similar topol. display of residues of the N-terminal helix important for PTH2 receptor activation. The C-terminal helix of TIP39 differs from that of PTH with respect to size and amphipathicity, suggesting an altered mode of binding for TIP39, consistent with the receptor chimera and ligand truncation studies presented in the accompanying paper. The structural characterization of TIP39 also provides some insight into the lack of affinity of this novel ligand for the PTH1 receptor.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 192 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2000:641937 HCAPLUS

DOCUMENT NUMBER: 133:276495

TITLE: Molecular determinants of tuberoinfundibular peptide of 39 residues (TIP39) selectivity for the parathyroid hormone-2 (PTH2) receptor. N-terminal truncation of TIP39 reverses PTH2 receptor/PTH1 receptor binding selectivity

AUTHOR(S): Hoare, Sam R. J.; Clark, Janet A.; Usdin, Ted B.

CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National Institute of Mental Health, Bethesda, MD, 20892, USA

SOURCE: Journal of Biological Chemistry (2000), 275(35), 27274-27283

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Tuberoinfundibular peptide of 39 residues (TIP39) and the parathyroid hormone-2 (PTH2) receptor form part of an extended family of related signaling mols. that includes the PTH1 receptor, which responds to PTH and PTH-related protein. TIP39 does not appreciably activate the PTH1 receptor, but in this study it is shown to bind the receptor with moderate affinity (59 nM). In this study, the authors investigated the mol. determinants of both ligand and receptor for the PTH2 receptor selectivity of TIP39 and quant. evaluated the role of mol. elements in the binding of TIP39 to the PTH2 and PTH1 receptors. A chimeric receptor composed of the N-terminal extracellular domain of the PTH1 receptor and the remainder (juxtamembrane domain) of the PTH2 receptor (P2-NP1) was fully activated by TIP39 ( $E_{max}$  = 98% of the rPTH-(1-34),  $E_{max}$ ,  $EC_{50}$  = 2.0 nM). This receptor chimera bound TIP39 with an equivalent affinity to the wild-type PTH2 receptor (2.3 and 2.0 nM, resp.). The reciprocal chimeric receptor (P1-NP2) was not activated by TIP39 and bound the ligand with an affinity equivalent to that of the PTH1 receptor. Thus, the juxtamembrane receptor domain specifies the signaling and binding selectivity of TIP39 for the PTH2 receptor over the PTH1 receptor. Removing six N-terminal residues of TIP39 eliminated activation of the PTH2 receptor and reduced binding affinity 70-fold. In contrast, this truncation increased affinity for the PTH1 receptor 10-fold, reversing the PTH2/PTH1 receptor binding selectivity and resulting in a high affinity interaction of TIP-(7-39) with the PTH1 receptor (6 nM). These findings can be explained by a strong interaction between the N-terminal region of TIP39 and the juxtamembrane domain of the PTH2 receptor, with the corresponding domain of the PTH1 receptor acting as a selectivity barrier against high affinity binding of TIP39. As a result, TIP-(7-39) is a highly potent, selective antagonist for the PTH1 receptor.



REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 193 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:615887 HCAPLUS

DOCUMENT NUMBER: 131:318101

TITLE: Comparison of rat and human parathyroid  
hormone 2 (PTH2) receptor  
activation: PTH is a low potency partial agonist at  
the rat PTH2 receptor

AUTHOR(S): Hoare, Sam R. J.; Bonner, Tom I.; Usdin, Ted B.

CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National  
Institute of Mental Health, Bethesda, MD, 20892-4094,  
USA

SOURCE: Endocrinology (1999), 140(10), 4419-4425  
CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The human PTH2 receptor, expressed in tissue culture cells, is selectively  
activated by PTH. Detailed investigation of its anatomical and cellular  
distribution has been performed in the rat. It is expressed by neurons in  
a number of brain nuclei; by endocrine cells that include pancreatic islet  
somatostatin cells, thyroid parafollicular cells, and peptide secreting  
cells in the gastrointestinal tract; and by cells in the vasculature and  
heart. The physiol. role of the PTH2 receptor expressed by these cells  
remains to be determined. All pharmacol. studies performed to date have used  
the human receptor. We have now isolated a complementary DNA including  
the entire coding sequence of the rat PTH2 receptor and compared its  
pharmacol. profile with that of the human PTH2 receptor when each is  
expressed in COS-7 cells. PTH-based peptides, including rat PTH(1-84),  
rat PTH(1-34), and human PTH(1-34), have low potency at the rat PTH2  
receptor for stimulation of adenylyl cyclase ( $EC_{50} = 19-140$  nM). When  
compared with the effect of a bovine hypothalamic extract, PTH-based peptides  
are partial agonists at the rat PTH2 receptor. This suggests that PTH is  
unlikely to be a physiol. important endogenous ligand for the PTH2  
receptor. A peptide homologous to an activity detected in a bovine  
hypothalamic extract is a good candidate for the endogenous PTH2 receptor  
ligand.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 194 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:403450 HCAPLUS

DOCUMENT NUMBER: 131:153922

TITLE: Distribution of the parathyroid  
hormone 2 receptor in rat:  
immunolocalization reveals expression by several  
endocrine cells

AUTHOR(S): Usdin, Ted B.; Hilton, Joanne; Vertesi, Tunde; Harta,  
Gyongyi; Segre, Gino; Mezey, Eva

CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental  
Health, National Institutes of Health, Bethesda, MD,  
20892, USA

SOURCE: Endocrinology (1999), 140(7), 3363-3371  
CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The PTH2 receptor is a G protein-coupled receptor selectively activated by  
PTH. The authors studied the receptor distribution to guide the  
investigation of its physiol. function. The authors have now generated an  
antibody from a C-terminal peptide sequence of the PTH2 receptor and used  
this to study its cellular distribution. Labeling with the antibody

identified a number of endocrine cells expressing the PTH2 receptor, including thyroid parafollicular cells, pancreatic islet D cells, and some gastrointestinal peptide synthesizing cells. There was complete overlap of PTH2 receptor labeling with somatostatin in pancreatic islets, and partial overlap with somatostatin in thyroid parafollicular cells and in the gastrointestinal tract. Furthermore, observations made previously by in situ hybridization histochem., including expression throughout the cardiovascular system, as well as by discrete populations of cells within the gastrointestinal tract and reproductive system were confirmed. These data suggest a broad role for the PTH2 receptor, especially within the endocrine

system, and provide a basis for exptl. exploration of its physiol.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 195 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1998:122181 HCAPLUS

DOCUMENT NUMBER: 128:239559

TITLE: Transmembrane residues together with the amino terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide

AUTHOR(S): Turner, Paul R.; Mefford, Suzanne; Bambino, Tom; Nissenson, Robert A.

CORPORATE SOURCE: Endocrine Unit, Veterans Affairs Medical Center and the Departments of Medicine and Physiology, University of California, San Francisco, CA, 94121, USA

SOURCE: Journal of Biological Chemistry (1998), 273(7), 3830-3837

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The mechanisms of ligand binding and receptor activation for G-protein-coupled receptors in the secretin/parathyroid hormone (PTH) receptor subfamily are not understood. The PTH1 receptor (PTH1R) signals in response to both PTH and parathyroid hormone-related peptide (PTHrP), whereas the PTH2 receptor (PTH2R) responds only to PTH, not to PTHrP. To locate PTHrP discriminatory domains in the PTH2R, we generated PTH1R/PTH2R chimeras in which the extracellular amino-terminal domains were exchanged. Production of cAMP in response to 1  $\mu$ M PTHrP or PTH was identical in cells expressing the PTH1R with the PTH2R amino terminus and in cells expressing the PTH2R with the PTH1R amino terminus. The ability of the chimeric receptor with the PTH2R amino terminus to respond fully to PTHrP showed that the body of the PTH2R must contain sites that limit the response to PTHrP. Mutations to PTH1R sequence were therefore made in each of the seven transmembrane domains of the PTH2R. Mutations in transmembrane domains 3 and 7 resulted in receptors able to respond to PTHrP. Thus, residues in more than one domain form a barrier or filter, allowing the receptor to discriminate between different ligands.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 196 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1998:86428 HCAPLUS

DOCUMENT NUMBER: 128:213457

TITLE: Multiple regions of ligand discrimination revealed by analysis of chimeric parathyroid hormone 2 (PTH2) and PTH/PTH-related peptide (PTHrP) receptors

AUTHOR(S): Clark, J. A.; Bonner, T. I.; Kim, A. S.; Usdin, T. B.  
CORPORATE SOURCE: Section Genetics, Natl. Inst. Mental Health, Bethesda, MD, 20892-4090, USA

SOURCE: Molecular Endocrinology (1998), 12(2), 193-206

CODEN: MOENEN; ISSN: 0888-8809

PUBLISHER: Endocrine Society  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB PTH and PTH-related peptide (PTHrP) bind to the PTH/PTHrP receptor and stimulate cAMP accumulation with similar efficacy. Only PTH activates the PTH2 receptor. To examine the structural basis for this selectivity, we analyzed receptor chimeras in which the amino terminus and third extracellular domains of the two receptors were interchanged. All chimeric receptors bound radiolabeled PTH with high affinity. Transfer of the PTH2 receptor amino terminus to the PTH/PTHrP receptor eliminated high-affinity PTHrP binding and significantly decreased activation by PTHrP. A PTH/PTHrP receptor N terminus modified by deletion of the non-homologous E2 domain transferred weak PTHrP interaction to the PTH2 receptor. Introduction of the PTH2 receptor third extracellular loop into the PTH/PTHrP receptor increased the EC50 for PTH and PTHrP, while preserving high-affinity PTH binding and eliminating high-affinity PTHrP binding. Similarly, transfer of the PTH/PTHrP receptor third extracellular loop preserved high-affinity PTH binding by the PTH2 receptor but decreased its activation. Return of Gln440 and Arg394, corresponding residues in the PTH/PTHrP and PTH2 receptor third extracellular loops, to the parent residue restored function of these receptors. Simultaneous interchange of wild-type amino termini and third extracellular loops eliminated agonist activation but not binding for both receptors. Function was restored by elimination of the E2 domain in the receptor with a PTH/PTHrP receptor N terminus and return of Gln440/Arg394 to the parent sequence in both receptors. These data suggest that the amino terminus and third extracellular loop of the PTH2 and PTH/PTHrP receptors interact similarly with PTH, and that both domains contribute to differential interaction with PTHrP.

REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 197 OF 221 HCAPLUS. COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1997:272579 HCAPLUS

DOCUMENT NUMBER: 126:246878

TITLE: The parathyroid hormone-2  
receptor: current status

AUTHOR(S): Usdin, Ted B.

CORPORATE SOURCE: Section on Genetics, National Institute of Mental  
Health, Bethesda, MD, 20892, USA

SOURCE: Experimental and Molecular Medicine (1997), 29(1),  
13-17

CODEN: EMMEF3

PUBLISHER: Korean Society of Medical Biochemistry and Molecular  
Biology

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review, with 32 refs. G-protein coupled receptors form a large superfamily of plasma membrane proteins which serve a variety of signal transduction roles. New receptors continue to be identified. Based on sequence homol. the superfamily can currently be divided into 3 families, the rhodopsin family which includes the vast majority of identified receptors, and the secretin and metabotropic glutamate receptor families which share a general architecture with each other and the rhodopsin family but no obvious sequence identity. Screening for addnl. members of the secretin family led to the identification of the parathyroid hormone-2 (PTH2) receptor. Ligand recognition by the PTH2 receptor partially overlaps that of the PTH/parathyroid hormone-related peptide (PTHrP) receptor. This has facilitated structure-function anal. of ligands for these receptors. The physiol. role of the PTH2 receptor is under investigation but its distribution suggests that it may be a neurotransmitter receptor and could participate in modulation of a number of organ systems. The relative abundance of PTH2

receptor mRNA in the brain and the inability to detect mRNA encoding PTH, its only currently identified ligand, suggest the existence of another endogenous ligand, for which evidence has recently been obtained.

L7 ANSWER 198 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1997:70497 HCAPLUS

DOCUMENT NUMBER: 126:153027

TITLE: Evidence for a parathyroid hormone  
-2 receptor selective ligand in the  
hypothalamus

AUTHOR(S): Usdin, Ted B.

CORPORATE SOURCE: National Institute Mental Health, Bethesda, MD, 20892,  
USA

SOURCE: Endocrinology (1997), 138(2), 831-834

CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The PTH2 receptor is expressed in several brain nuclei but we have been unable to detect mRNA encoding PTH, which is the only known ligand for the PTH2 receptor, in the brain. We now have evidence for a PTH2 receptor selective ligand in an acid-acetone extract made from bovine hypothalamus. The partially purified extract activates the PTH2 receptor more effectively than it activates the PTH/PTHrP receptor, while PTH activates these two receptors at similar concentration. The activity appears immunol. distinct from PTH and its effect is potently antagonized by [D-Trp12]bPTH(7-34). These data provide evidence for a biol. active peptide, which may be related to PTH, and which is a potential new neurotransmitter or hormone.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 199 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1996:742756 HCAPLUS

DOCUMENT NUMBER: 126:42793

TITLE: Truncation of the amino terminus of PTH alters its  
anabolic activity on bone in vivo

AUTHOR(S): Hilliker, S.; Wergedal, J. E.; Gruber, H. E.; Bettica,  
P.; Baylink, D. J.

CORPORATE SOURCE: Jerry L. Pettis Veterans' Administration Medical  
Center, Loma Linda, CA, 92350, USA

SOURCE: Bone (New York) (1996), 19(5), 469-477

CODEN: BONEDL; ISSN: 8756-3282

PUBLISHER: Elsevier

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In vitro studies of parathyroid hormone (PTH) structure and function have suggested that the anabolic effect of PTH on bone requires the presence of amino acid residues 28-34 (domains for protein kinase C activation and mitogenic activity), but not amino acid residues 1-7 (adenylate cyclase activation domain). The authors have tested this hypothesis with in vivo studies of human PTH (hPTH) analogs. Serum biomarkers and selected histomorphometric parameters of bone formation and resorption were assessed in adult, female, Sprague-Dawley rats following 19 daily injections of vehicle, 10 µg/kg body weight (bw) of hPTH(1-38), or a dose range of 10, 40, and 100 µg/100 g bw of hPTH(2-38) or hPTH(3-38). Treatment with hPTH(1-38) increased serum osteocalcin, the percentage of osteoblast surface, percentage of osteoid surface, percentage of bone volume, trabecular thickness, and bone formation rate, while it decreased the percentage of osteoclast surface. The hPTH(2-38) fragment exhibited 10%-25% of the in vivo anabolic activity of hPTH(1-38), while it had no effect on the percentage of osteoclast surface. The hPTH(3-38) fragment exhibited no biol. activity on bone. In contrast, serum INS-PTH (intact-N-terminal specific PTH) levels were similarly and significantly increased above control in rats treated with hPTH(1-38), hPTH(2-38), or



hPTH(3-38) at the same dose. This preliminary finding suggests that the differential activity of these peptides on bone is not due to differences in the circulating level of immunoreactive PTH (intact and N-terminal fragments of PTH from endogenous and exogenous sources) several hours after PTH injection. However, the authors can draw no conclusion regarding the relative clearance rates of these peptides. Last, because hPTH(3-38) was without any detectable biol. activity on rat bone in vivo, its mitogenic activity was confirmed in two osteoblast-like cell lines. In summary, the anabolic effect of hPTH(1-38) on bone in vivo was (1) diminished by removal of amino acid residue 1, and (2) abolished by the removal of amino acid residues 1 and 2. Although these findings suggest that the therapeutic benefits of exogenous PTH administration may depend upon activation of not only protein kinase C, but also adenylate cyclase, they do not rule out a differential PTH response due to other causes, e.g., metabolic inactivation.

L7 ANSWER 200 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1996:580502 HCAPLUS

DOCUMENT NUMBER: 125:293602

TITLE: Distribution of parathyroid hormone  
-2 receptor messenger ribonucleic acid in  
rat

AUTHOR(S): Usdin, Ted B.; Bonner, Tom I.; Harta, Gyongyi; Mezey,  
Eva

CORPORATE SOURCE: Lab. Cell Biol., Natl. Inst. Health, Bethesda, MD,  
20892, USA

SOURCE: Endocrinology (1996), 137(10), 4285-4297  
CODEN: ENDOAO; ISSN: 0013-7227

PUBLISHER: Endocrine Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We obtained a cDNA encoding the rat PTH2 receptor and used it to study the distribution of the PTH2 receptor using in situ hybridization histochem. PTH2 receptor mRNA is abundantly expressed in arterial and cardiac endothelium and at lower levels in vascular smooth muscle. It is also abundant in the lung, both within bronchi and in the parenchyma, and is present within the exocrine pancreas. It is expressed by sperm in the head of the epididymis. A small number of cells associated with the vascular pole of renal glomeruli express the receptor. These data suggest that the PTH2 receptor may be responsible for PTH effects in a number of physiol. systems.

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
L2 5 S L1 AND (MAPKAP-2 (W)KINASE?)  
L3 4 DUP REM L2 (1 DUPLICATE REMOVED)  
L4 119389 S PARATHYROID (W)HORMONE##  
L5 6 S L1 AND L4  
L6 5 DUP REM L5 (1 DUPLICATE REMOVED)  
L7 221 S PARATHYROID (W)HORMONE-2  
L8 1 S L1 AND L7  
L9 68 S IMMUN? AND L7  
L10 1 S L1 AND L9  
L11 369433 S SKELETAL (W)MUSCLE  
L12 387 S L4 AND L11  
L13 0 S L7 AND L11

=> s l7 and (isolat? or purif?)

L14 76 L7 AND (ISOLAT? OR PURIF?)

=> dup rem l14

PROCESSING COMPLETED FOR L14

L15 33 DUP REM L14 (43 DUPLICATES REMOVED)

=> d 1-33 ibib ab

L15 ANSWER 1 OF 33 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 2006249062 IN-PROCESS  
DOCUMENT NUMBER: PubMed ID: 16570212  
TITLE: The distribution and neurochemistry of the  
parathyroid hormone 2 receptor  
in the rat hypothalamus.  
AUTHOR: Dobolyi Arpad; Irwin Sarah; Wang Jing; Usdin Ted Bjorn  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental  
Health, Bethesda, MD 20892, USA.  
SOURCE: Neurochemical research, (2006 Feb) Vol. 31, No. 2, pp.  
227-36. Electronic Publication: 2006-03-29.  
Journal code: 7613461. ISSN: 0364-3190.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals  
ENTRY DATE: Entered STN: 5 May 2006  
Last Updated on STN: 3 Jun 2006

AB This study reports the distribution of parathyroid  
hormone 2 receptor (PTH2R)-immunoreactive fibers in the  
hypothalamus using fluorescent amplification immunocytochemistry. The  
pattern of immunolabeling is strikingly similar to that of  
tuberoinfundibular peptide of 39 residues (TIP39), a peptide recently  
purified from bovine hypothalamus and proposed to be a ligand of  
the PTH2R based on pharmacological data. To investigate the anatomical  
basis of suggestions that TIP39 affects the secretion of several  
hypophysiotropic hormones we performed double-labeling studies and found  
that only somatostatin fibers contain PTH2R in the median eminence, which  
suggests that somatostatin release could be directly regulated via the  
PTH2R. However, several hypothalamic nuclei projecting to the median  
eminence contain a high density of both TIP39 and PTH2R fibers and  
terminals. We report here, that the PTH2R terminals also contain  
vesicular glutamate transporter-2, and suggest that TIP39 terminals are  
ideally positioned to modulate glutamatergic influences on  
hypophysiotropic neurons.

L15 ANSWER 2 OF 33 MEDLINE on STN  
ACCESSION NUMBER: 2004133701 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 14684608  
TITLE: Identification and characterization of two parathyroid  
hormone-like molecules in zebrafish.  
AUTHOR: Gensure Robert C; Ponugoti Bhaskar; Gunes Yasemin; Papasani  
Madhusudhan R; Lanske Beate; Bastepe Murat; Rubin David A;  
Juppner Harald  
CORPORATE SOURCE: Endocrine Unit, Massachusetts General Hospital, Harvard  
Medical School, Boston 02114, USA.  
CONTRACT NUMBER: DK11794 (NIDDK)  
DK60513 (NIDDK)  
SOURCE: Endocrinology, (2004 Apr) Vol. 145, No. 4, pp. 1634-9.  
Electronic Publication: 2003-12-18.  
Journal code: 0375040. ISSN: 0013-7227.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
OTHER SOURCE: GENBANK-AY275669; GENBANK-AY275670; GENBANK-AY302221

ENTRY MONTH: 200404  
ENTRY DATE: Entered STN: 18 Mar 2004  
Last Updated on STN: 17 Apr 2004  
Entered Medline: 16 Apr 2004

AB Zebrafish (*Danio rerio*) have receptors homologous to the human PTH (hPTH)/PTHrP receptor (PTH1R) and PTH-2 receptor (PTH2R) and an additional receptor (PTH3R) with high homology to the PTH1R. To find natural ligands for zPTH1R and zPTH3R, we searched the zebrafish genomic database and discovered two distinct regions that, when translated (zPTH1 and zPTH2), showed high homology to hPTH. Isolation of cDNAs and determination of the intron/exon boundaries revealed genomic structures which were similar to known PTHs. Peptides consisting of the first 34 amino acids after the pre- and prosequences of the zebrafish PTHs (zPTHs) were synthesized and were shown to be fully active at the hPTH1R. zPTH2(1-34) was, however, approximately 30-fold less potent at the zPTH1R than hPTH(1-34), hPTHrP(1-36), and zPTH1(1-34). When tested with zPTH3R, zPTH1(1-34) and hPTHrP(1-36) showed similar potencies, whereas the potency of zPTH2(1-34) was moderately (3-fold) reduced. To determine whether other fishes have multiple PTHs, we searched the genomic database of the Japanese pufferfish (*Takifugu rubripes*) and identified zPTH1 and zPTH2 homologs. Phylogenetic analysis showed that PTHs from zebrafish and pufferfish are more closely related to each other than to known mammalian PTH homologs or to PTHrP and tuberoinfundibular peptide of 39 residues. This is consistent with evolution of two teleost PTH-like peptides occurring after the evolutionary divergence between fishes and mammals. Overall, the PTH system appears more complex in fishes than in mammals, providing evidence of continued evolution in nontetrapod species. The availability of multiple forms of fish PTH and their receptors provide additional tools for PTH ligand/receptor structure-function studies.

L15 ANSWER 3 OF 33 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2003-10866 BIOTECHDS

TITLE: Producing antibodies to a three-dimensional epitope of a bioactive human parathyroid hormone for diagnosing or treating e.g., hyperparathyroidism by immunizing an animal with the hormone and recovering the antibodies;  
for use in gene therapy, recombinant vaccine and nucleic acid vaccine preparation

AUTHOR: HUTCHISON J S

PATENT ASSIGNEE: QUEST DIAGNOSTICS INVESTMENTS INC

PATENT INFO: WO 2003003986 16 Jan 2003

APPLICATION INFO: WO 2002-US21356 3 Jul 2002

PRIORITY INFO: US 2001-898398 3 Jul 2001; US 2001-898398 3 Jul 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-229379 [22]

AB DERWENT ABSTRACT:

NOVELTY - Producing antibodies to a three-dimensional epitope of a bioactive human parathyroid hormone comprises: (a) immunizing an animal with the bioactive human parathyroid hormone; and (b) recovering antibodies from the animal. The antibodies specifically recognize the three-dimensional structure of the bioactive human parathyroid hormone.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a method for producing antibodies that recognize and bind the bioactive, three-dimensional epitope of parathyroid hormone; (2) an antibody that recognizes and binds the bioactive, three-dimensional epitope of parathyroid hormone; (3) a therapeutic composition comprising the antibody; (4) a kit comprising the antibody; (5) a method for detecting bioactive human parathyroid hormone in a sample; and (6) an immunoassay comprising the antibody.

BIOTECHNOLOGY - Preferred Method: Producing antibodies to a three-dimensional epitope of a bioactive human parathyroid hormone further comprises, immunizing the animal with the human parathyroid hormone a second time before recovering the antibodies from the animal;

and isolating the antibodies. The bioactive human parathyroid hormone is coupled to a carrier, which is keyhole limpet hemocyanin. It comprises 84-amino acid sequence. The antibodies are isolated by affinity chromatography or screening the antibodies with fragments of the human parathyroid hormone linked to a solid phase. Producing antibodies that recognize and bind the bioactive, three-dimensional epitope of parathyroid hormone comprises, immunizing an animal with the bioactive human parathyroid hormone; immunizing the animal with the human parathyroid hormone a second time; and recovering antibodies from the animal. The bioactive, three-dimensional epitope is the amino terminus of the parathyroid hormone. The method further comprises isolating the recovered antibodies. The antibodies are isolated by fragments of parathyroid hormone coupled to a solid phase. The fragments comprise amino acids 1-13, 13-34 or 39-84 of the 84-amino acid sequence. Detecting bioactive human parathyroid hormone in a sample comprises exposing the sample to an antibody that recognizes and binds the bioactive three-dimensional epitope of parathyroid hormone; and detecting the antibody-hormone complex. The method further comprises exposing the antibody-hormone complex to another antibody that recognizes and binds parathyroid hormone before detecting the antibody-hormone complex. The method also comprises exposing the sample to a capture antibody that recognizes and binds the bioactive three-dimensional epitope of parathyroid hormone; exposing the capture antibody-hormone complex to a detection antibody that binds a different epitope than the capture antibody; and detecting the antibody-hormone complex. The sample is from a patient with hyperparathyroidism or hypoparathyroidism. Preferred Antibody: The antibody recognizes a peptide comprising an amino acid sequence from Ser in position 1 to Lys in position 13 of the 84-amino acid sequence. The antibody reduces adenylate cyclase activity by binding to the bioactive portion of the parathyroid hormone. It is a polyclonal, monoclonal or humanized antibody or an antibody fragment. It is coupled to a detectable marker. Preferred Composition: The composition comprises the antibody and a carrier. Preferred Kit: The kit further comprises tools for obtaining a biological sample containing parathyroid hormone from the patient. The antibody is coupled with a detectable label, comprising chemiluminescent, fluorescent, radioactive or enzymatic markers, preferably acridinium ester.

ACTIVITY - Antithyroid; Immunostimulant. Test details are described but no results given.

MECHANISM OF ACTION - Vaccine; Gene therapy.

USE - The method is useful for manufacturing a medicament for diagnosing or treating hyperparathyroidism or hypoparathyroidism.

ADMINISTRATION - Dosage comprises 1 ng to 10 mg per kg body weight. The composition is administered via oral or parenteral route. (69 pages)

L15	ANSWER 4 OF 33	MEDLINE on STN	DUPLICATE 2
ACCESSION NUMBER:	2003001658	MEDLINE	
DOCUMENT NUMBER:	PubMed ID: 12508326		
TITLE:	Expression and distribution of tuberoinfundibular peptide of 39 residues in the rat central nervous system.		
AUTHOR:	Dobolyi Arpad; Palkovits Miklos; Usdin Ted Bjorn		
CORPORATE SOURCE:	Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland 20892-4094, USA.		
SOURCE:	The Journal of comparative neurology, (2003 Jan 20) Vol. 455, No. 4, pp. 547-66. Journal code: 0406041. ISSN: 0021-9967.		
PUB. COUNTRY:	United States		
DOCUMENT TYPE:	Journal; Article; (JOURNAL ARTICLE)		
LANGUAGE:	English		
FILE SEGMENT:	Priority Journals		
ENTRY MONTH:	200303		
ENTRY DATE:	Entered STN: 2 Jan 2003 Last Updated on STN: 20 Mar 2003 Entered Medline: 19 Mar 2003		



AB    Tuberoinfundibular peptide of 39 residues (TIP39) has been recently purified and identified as a selective ligand for the parathyroid hormone 2 receptor. As a next step toward understanding its functions, we report the expression and distribution of TIP39 in the rat central nervous system. In situ hybridization histochemistry and immunocytochemistry revealed TIP39-containing cell bodies in three distinct areas. The major one comprises the subparafascicular area posterior through the intralaminar nucleus of the thalamus; a second is the medial paralemniscal nucleus at the pontomesencephalic junction; and a third is in the dorsal and dorsolateral hypothalamic areas, which contained a few, scattered cell bodies. We found, in contrast to the highly restricted localization of TIP39-containing cell bodies, a much more widespread localization of TIP39-containing fibers. The highest density of fibers was observed in limbic areas such as the septum, the amygdala, and the bed nucleus of the stria terminalis; in areas involved in endocrine regulation, such as the hypothalamic dorsomedial, paraventricular, periventricular, and arcuate nuclei; in auditory areas, such as the ectothalamic and temporal cortices, inferior colliculus, medial geniculate body, and some of the nuclei of the superior olivary complex; and in the dorsolateral funiculus of the spinal cord. The localization of TIP39-containing nuclei and fibers provides an anatomical basis for previously demonstrated endocrine and nociceptive effects of TIP39 and suggests additional functions for TIP39, one apparent candidate being the regulation of auditory information processing.

L15    ANSWER 5 OF 33                    MEDLINE on STN                    DUPLICATE 3  
ACCESSION NUMBER:    2003306019                    MEDLINE  
DOCUMENT NUMBER:    PubMed ID: 12834174  
TITLE:                    The parathyroid hormone-2  
                                 receptor is expressed on human leukocytes and  
                                 down-regulated in hyperparathyroidism.  
AUTHOR:                    Seeliger S; Hausberg M; Eue I; Usdin T; Rahn K H; Kosch M  
CORPORATE SOURCE:    Department of Paediatrics, University Hospital Munster,  
                                 Germany.  
SOURCE:                    Clinical nephrology, (2003 Jun) Vol. 59, No. 6, pp. 429-35.  
                                 Journal code: 0364441. ISSN: 0301-0430.  
PUB. COUNTRY:                    Germany: Germany, Federal Republic of  
DOCUMENT TYPE:                    Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE:                    English  
FILE SEGMENT:                    Priority Journals  
ENTRY MONTH:                    200310  
ENTRY DATE:                    Entered STN: 2 Jul 2003  
                                 Last Updated on STN: 4 Oct 2003  
                                 Entered Medline: 3 Oct 2003

AB    BACKGROUND: Parathyroid hormone (PTH) has specific effects on function, migration and proliferation of human leukocytes. These effects may contribute to accelerated atherosclerosis and impaired immune response observed in patients with renal insufficiency. Recently, a new G protein-coupled receptor with substantial implications for vascular function--the PTH2 receptor (PTH2-R)--has been identified, however, expression and distribution in humans and a possible regulation has not yet been studied. We therefore investigated the expression of the PTH2 receptor on human leukocytes in healthy subjects and in patients with hyperparathyroidism. METHODS: PTH2 receptor expression was quantified by flow cytometry (FACS) analysis on monocytes, lymphocytes and granulocytes that were isolated from peripheral blood (hypotonic density gradient centrifugation) and by immunohistochemistry using a specific alpha-PTH2-R antibody produced in rabbit. Results of 22 patients with hyperparathyroidism (12 renal allograft recipients, 10 hemodialysis patients, mean age 43 +/- 8 years) were compared to 22 age and sex-matched healthy controls. RESULTS: Mean relative antigen density of the PTH2 receptor and percentage of positive cells in healthy subjects was 19 +/- 5 and 90 +/- 6% on granulocytes, 5 +/- 2 and 55 +/- 19% on monocytes, and 24 +/- 7 and 21 +/- 7% on lymphocytes. In patients with hyperparathyroidism,

mean antigen density was significantly lower on granulocytes and monocytes (17 +/- 4% and 3 +/- 1%, p < 0.01, respectively). The percentage of positive cells and mean expression on lymphocytes was not significantly different. A significant and inverse correlation was found between plasma PTH concentrations and the mean PTH2 receptor expression on granulocytes (r = -0.41, p < 0.05). CONCLUSIONS: The PTH2 receptor is expressed on human granulocytes and--to a lesser degree--on monocytes and lymphocytes. In patients with hyperparathyroidism the PTH2 receptor is down-regulated as function of plasma PTH levels.

L15 ANSWER 6 OF 33 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 2003050743 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12559132  
 TITLE: Tuberoinfundibular peptide of 39 residues (TIP39): molecular structure and activity for parathyroid hormone 2 receptor.  
 AUTHOR: Della Penna K; Kinose F; Sun H; Koblan K S; Wang H  
 CORPORATE SOURCE: Department of Molecular Pharmacology, Merck Research Laboratories, West Point, PA 19486, USA.  
 SOURCE: Neuropharmacology, (2003 Jan) Vol. 44, No. 1, pp. 141-53. Journal code: 0236217. ISSN: 0028-3908.  
 PUB. COUNTRY: England: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200304  
 ENTRY DATE: Entered STN: 2 Feb 2003  
 Last Updated on STN: 1 May 2003  
 Entered Medline: 30 Apr 2003  
 AB The neuropeptide TIP39 was recently purified from bovine hypothalamus based on the ability of the peptide to activate the parathyroid hormone 2 receptor (PTH2R) ( Nat. Neurosci. 2 (1999) 941). PTH2R is abundantly expressed in the nervous system, and its expression pattern suggests that it may play a role in modulation of pituitary function and in nociception. Towards understanding the physiological role of TIP39 and PTH2R, we cloned human, mouse and rat TIP39 gene. Our results revealed that: (1) the mature peptide is processed from a precursor; (2) TIP39 peptide is highly conserved among species; and (3) TIP39 from all species activates adenylyl cyclase and elevates intracellular calcium levels through PTH2R. We also defined and compared the structure-activity relationship of TIP39 on both activation of adenylyl cyclase and calcium mobilization pathways through PTH2R, finding common and differential determinants of TIP39 that are required for these pathways. Furthermore, we observed that TIP39 elevates intracellular calcium levels in primary dorsal root ganglion neurons whereas the peptide inactive on PTH2R do not, suggesting that TIP39 may activate these neurons important for nociception in vivo through PTH2R-dependent mechanisms.

L15 ANSWER 7 OF 33 MEDLINE on STN DUPLICATE 5  
 ACCESSION NUMBER: 2002713991 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12475607  
 TITLE: Emerging functions for tuberoinfundibular peptide of 39 residues.  
 AUTHOR: Usdin Ted B; Dobolyi Arpad; Ueda Hiroshi; Palkovits Miklos  
 CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, National Institutes of Health, Building 36/Room 3D06, 36 Convent Drive, Bethesda, MD 20892-4094, USA.. usdin@codon.nih.gov  
 SOURCE: Trends in endocrinology and metabolism: TEM, (2003 Jan) Vol. 14, No. 1, pp. 14-9. Ref: 27 Journal code: 9001516. ISSN: 1043-2760.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200307  
ENTRY DATE: Entered STN: 17 Dec 2002  
Last Updated on STN: 26 Jul 2003  
Entered Medline: 25 Jul 2003

AB Tuberoinfundibular peptide of 39 residues (TIP39), a neuropeptide recently purified from the hypothalamus, appears to be an endogenous ligand for the parathyroid hormone 2 (PTH2) receptor. PTH2 receptors are present in several central nervous system and peripheral areas and are particularly concentrated in the hypothalamus, limbic areas and the outer layers of the spinal cord dorsal horn. TIP39-containing neuronal cell bodies have been identified in the subparafascicular area and the medial paralemniscal nucleus, two brainstem regions that project widely through the entire neuraxis. Treatment of hypothalamic explants with TIP39, and intraventricular injection of the peptide, suggest that it might stimulate hypothalamic-releasing factor secretion. Injection of TIP39, and sequestration of endogenous TIP39 by intrathecal injection of an antibody to TIP39, have provided evidence that it is involved in some aspects of pain sensitivity. Thus, TIP39 might be a new neuromodulator.

L15 ANSWER 8 OF 33 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
ACCESSION NUMBER: 2004:205393 BIOSIS  
DOCUMENT NUMBER: PREV200400205920  
TITLE: The localization and projections of TIP39 neurons suggest limbic, endocrine, auditory and nociceptive functions of the neuropeptide.  
AUTHOR(S): Dobolyi, A. [Reprint Author]; Palkovits, M. [Reprint Author]; Bodnar, I. [Reprint Author]; Usdin, T. B. [Reprint Author]  
CORPORATE SOURCE: Lab. of Genet., Natl. Inst. of Mental Hlth., NIH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 889.23.  
<http://sfn.scholarone.com>. e-file.  
Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.  
DOCUMENT TYPE: Conference; (Meeting)  
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AB Tuberoinfundibular peptide of 39 residues (TIP39) was recently purified as a parathyroid hormone 2 receptor ligand. Our previous in situ hybridization and RT-PCR studies showed that TIP39 neurons are restricted to two distinct nervous system areas, one at the diencephalon-midbrain junction, and the other in the lateral pons. We have now investigated the precise localization and the projections of TIP39 neurons. One group of TIP39 cells extends from the caudal hypothalamus to the mesencephalic central gray. Most cells are near the midline, in the subparafascicular nucleus and medial to it and to the fasciculus retroflexus. Lesions here caused the disappearance of TIP39 fibers ipsilaterally in the limbic cortex, nucleus accumbens, lateral septum, bed nucleus of the stria terminalis, amygdaloid nuclei, thalamic paraventricular nucleus, and hypothalamic paraventricular, dorsomedial and arcuate nuclei. TIP39 terminals surround cell bodies and proximal dendrites of CRH cells in the paraventricular nucleus and are closely apposed to some GHRH and somatostatin cells. TIP39 cells in the lateral pons occupy an area between the pontine reticular nucleus and the nuclei of the lateral lemniscus, immediately dorsal to the rubrospinal tract in the caudal part of the medial paralemniscal nucleus. Lesions

here cause disappearance of TIP39 fibers ipsilaterally from the medial geniculate body, periaqueductal central gray, deep nuclei of the superior colliculus, inferior colliculus, nuclei of the lateral lemniscus, lateral parabrachial nucleus, locus coeruleus, trapezoid body, periolivary nuclei, and spinal cord. Our results suggest that the diencephalic TIP39 participates in limbic and endocrine functions, and that TIP39 in the lateral pons defines a new nucleus with previously undescribed auditory and nociceptive projections.

L15 ANSWER 9 OF 33 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
DUPLICATE 6

ACCESSION NUMBER: 2002-17835 BIOTECHDS

TITLE: Isolated polynucleotide encoding human TIP39  
peptide for identifying functional TIP39 and a test compound  
which modulates the activity of the peptide;  
vector-mediated recombinant protein gene transfer and  
expression in host cell for use in drug screening and gene  
therapy

AUTHOR: WANG H; KOBLAN K S; SUN H; DELLA PENNA K

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002033049 25 Apr 2002

APPLICATION INFO: WO 2000-US31954 17 Oct 2000

PRIORITY INFO: US 2000-241012 17 Oct 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-471397 [50]

AB DERWENT ABSTRACT:

NOVELTY - An isolated polynucleotide (I) comprising a fully defined 572 base pair sequence (S1) encoding a human tuberoinfundibular peptide with a fully defined 39 amino acid sequence, both given in the specification or a sequence that hybridizes to the complement of S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid molecule (II) comprising a coding region of a splice variant of human tuberoinfundibular peptide (TIP39) encoded by S1; (2) isolated mammalian, bacterial or amphibian oocyte cells (III) comprising (I); (3) an isolated human TIP39 (IV) encoded by (I); (4) a cell membrane preparation (V) comprising (IV) with a sequence (S2) of SLALADDAAFRERARLLAALERRHWLNSYMHKLLVLDAP produced by a cell that expresses a recombinant expression vector encoding (IV); (5) a recombinant expression sequence (VI) encoding (IV) and comprising S1 which expresses the peptide in a transformed culture of eukaryotic or prokaryotic cells; (6) a cell culture (VII) transformed with (VI); (7) an expression vector (VIII) comprising (II) operably linked to a regulatory nucleotide sequence that controls expression of (II) in a host cell; (8) following (M1) the progress of a therapeutic regime designed to alleviate a condition characterized by abnormal expression of a gene product of (I) involving assaying a sample to determine the level of a parameter selected from a polypeptide encoded by S1 and S2 between 2 time points to determine the effect of the therapeutic regime; (9) determining (M2) regression, progression or onset of a pathological disorder characterized by a dysfunctional signal transduction, involving incubating a sample with: (a) a complementary probe homologous to a fully defined 100 amino acid sequence given in the specification and determining binding between the probe and any complementary messenger ribonucleic acid (mRNA) that may be present in the sample or (b) a detectable probe that is specific for the gene product of (I); (10) a pharmaceutical composition (IX) comprising (IV); (11) monitoring (M3) the efficacy of an agent in correcting abnormal levels of (IV); (12) screening (M4) and identifying agonists of (IV), by contacting a cell line that expresses the human parathyroid hormone-2 (PTH2) receptor with a test compound in the presence and in the absence of (IV) and determining whether, in the presence of (IV), the test compound inhibits the binding of (IV) to a cell surface receptor in the cell line and if the test



compound mimics the cellular effects of (IV) on the cell line, in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of (IV) on the cell line; (13) screening (M5) and identifying antagonists of (IV), by contacting a cell line that expresses the human PTH2 receptor with a test compound in the presence of (IV), and determining whether the test compound inhibits the binding and cellular effects of (IV) on the cell line, in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of (IV) on the cell line; (14) screening (M6) for a disorder characterized by expression of a dysfunctional TIP39, coded for by a cDNA comprising a sequence of nucleotides substantially homologous to S1, involves: (a) contacting a sample from a subject believed to suffer from the disorder with an antibody specific for an expression product of S1, and determining binding between the antibody and the expression production as an indication of possible presence of the disorder in the subject; or (b) contacting a cDNA or mRNA containing sample from a subject with a nucleic acid hybridization probe which hybridizes to a cDNA molecule comprising S1, and determining binding of the hybridization probe to the cDNA or mRNA as an indication of possible presence of the disorder in the subject; (15) inhibiting (M7) binding of TIP39 to a cell presenting PTH2 receptor by adding an amount of an antagonist identified by M5 to a sample containing the cell in an amount sufficient to inhibit binding of the TIP39 to the cell; and (16) an antibody (X) that is specific for (IV).

WIDER DISCLOSURE - Disclosed are: (A) a plasmid containing (I); (B) a nucleic acid probe comprising nucleic acid molecules of sufficient length which specifically hybridize (I); (C) a diagnostic assay for detecting diseases related to mutations in (I); (D) a therapeutic composition comprising an immunological active or biological active fragments of (IV) or an antibody having affinity for (IV); (E) identifying cells that express (IV); (F) a nucleic acid having substantially the same sequence of S1; (G) a nucleic acid which differ from S1, but which has the same phenotype; (H) an antisense oligonucleotide having a sequence capable of binding specifically with any portion of mRNA that encodes (IV); (I) a transgenic non-human mammal capable of expressing (I); (J) diagnosing disease states characterized by abnormal signal transduction; and (K) a diagnostic system preferably in kit form comprising (I) in suitable packaging material.

BIOTECHNOLOGY - Preparation: (IV) is prepared by standard recombinant techniques. Preferred Nucleic Acid: (I) is genomic DNA, mRNA or cDNA. Preferred Method: In M2, the sample is a tissue. The probe is an antibody, where the antibody is labeled with a radioactive label or an enzyme. Preferred Antibody: (X) is a monoclonal antibody.

ACTIVITY - None given.

MECHANISM OF ACTION - Modulator of the endogenous signal transducing activity of a parathyroid hormone-2 (PTH2) receptor. No supporting data is given.

USE - (I) is useful for identifying functional TIP39, by introducing (I) into a suitable host cell that expresses a functional PTH2 receptor, and assaying for PTH2 receptor activity in the cells, or by introducing (I) into eukaryotic cells, and detecting PTH2 receptor activity in the cells, where the activity is mediated by a polypeptide encoded by the introduced nucleic acid molecule. (I) is useful for identifying DNA sequences encoding a TIP39, by probing a cDNA library or a genomic library with a labeled probe, preferably (I) comprising S1, and recovering from the library those sequences having a significant degree of homology relative to the probe. (I) is useful for detecting TIP39 messenger RNA in a biological sample by contacting all or part of S1 with the biological sample under conditions allowing a complex to form between the nucleic acid sequence and the messenger RNA, detecting the complexes, and determining the level of the messenger RNA. (I) is useful in a bioassay for identifying a test compound, which modulates the activity of TIP39. (IV) is useful for preventing or delaying onset of a condition associated with reduced or non-existent levels of (IV), for detecting a

binding partner for (IV) in a sample suspected of containing the binding partner, and for modulating the endogenous signal transducing activity of a PTH2 receptor in a mammal (claimed). (I) or (IV) is useful for in vitro purposes such as synthesis of DNA and manufacture of DNA vectors. (I), (IV) or (X) is useful as diagnostics, for distinguishing disease states caused by a dysfunctional endogenous TIP39 or PTH2 receptor, and for screening compounds in vitro to determine whether a compound functions as a potential agonist or antagonist to (IV). (X) is useful in immunohistochemistry techniques, for diagnostic and therapeutic applications, for purifying or detecting (IV), and for modulating the activity of (IV) in living animals or humans (all claimed).

EXAMPLE - A complementary deoxyribonucleic acid (cDNA) fragment specific to human tuberoinfundibular peptide (TIP39) was generated by polymerase chain reaction (PCR) amplification of human hypothalamus cDNA. The degenerate oligonucleotide primers (i) and (ii) were utilized to generate a fragment for plasmid subcloning. The resulting 98 base pair fragment was subcloned into the PCR II vector. Sequence analysis indicated that the fragment encoded a peptide that aligned with bovine TIP39 peptide between positions 5-36. The sequence information obtained from this clone was utilized to design the following oligonucleotide primer pairs, which yielded a PCR fragment of approximately 70 bp. Hw60 and KB01 primers were used to screen a human fetal brain stem cDNA library. Two identical clones were identified and sequenced. Hw37 TIGCIGA(T/C)GA(T/C)GCIGCITTCG (i); and Hw39 TCIA(A/G)IACIA(A/G)IA(A/G)IA(A/G)(C/T)TTGTGCAT (ii). (61 pages)

L15 ANSWER 10 OF 33 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-08555 BIOTECHDS

TITLE: New isolated nucleic acid molecule encoding a human  
mitogen-activated protein kinase activating protein kinase-2  
(MAPKAP-2), useful for treating immune-system related  
disorders, inflammation and arthritis;  
recombinant enzyme protein production and sense and  
antisense sequence for use in gene therapy

AUTHOR: LOGRASSO P; HAWKINS J; LISNOCK J M

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002090524 14 Nov 2002

APPLICATION INFO: WO 2002-US5670 25 Feb 2002

PRIORITY INFO: US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-111970 [10]

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if its is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the

cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5) Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to subtracting the product from  $X_a$ . The nucleic acid molecule has a sequence that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring



the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone-2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay. In (M13), the allelic variant is



encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound.

(M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis.

(M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

ADMINISTRATION - Administration is oral, topical or parenteral (e.g. intraarterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal). The dosage is 0.001-50 mg/kg, preferably 0.1-1.0 mg/kg.

EXAMPLE - No relevant example given. (150 pages)

ACCESSION NUMBER: 2003-02758 BIOTECHDS

TITLE: New isolated GPR54 polynucleotides and polypeptides, useful for preventing and/or treating disorders associated with an excess or deficiency of GPR54 protein, such as diabetes, pain, anxiety, depression and Alzheimer's disease;

vector-mediated recombinant protein gene transfer and expression in host cell for use in gene therapy

AUTHOR: LIU Q; CLEMENTS M; MCDONALD T P

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002059344 1 Aug 2002

APPLICATION INFO: WO 2001-US48333 14 Dec 2001

PRIORITY INFO: US 2000-256299 18 Dec 2000; US 2000-256299 18 Dec 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-666905 [71]

AB DERWENT ABSTRACT:

NOVELTY - A new isolated nucleic acid molecule (I) comprises a sequence of nucleotides that encodes a human G protein-coupled receptor (GPR54).

DETAILED DESCRIPTION - A new isolated nucleic acid molecule (I) comprises a sequence of nucleotides that encodes a human G protein-coupled receptor (GPR54), where the sequence of nucleotides are selected from: (i) a fully defined sequence of 1197 bp, given in the specification; (ii) a sequence that hybridizes under conditions of high stringency to the complement of the sequence in (i), and if it is DNA, a full complement or RNA, is identical to mRNA native to a human cell; or (iii) a degenerate sequence of (i) or (ii). INDEPENDENT CLAIMS are also included for the following: (1) isolated nucleic acid molecule comprising a coding region that encodes a splice variant of human GPR54 receptor, where it is encoded by a fully defined sequence of 1197 bp, given in the specification; (2) isolated polypeptide encoded by a nucleotide sequence that is a splice variant of an isolated nucleic acid molecule that encodes a protein with a fully defined sequence of 398 amino acids, given in the specification; (3) isolated cells comprising (I); (4) isolated human GPR54 receptor protein (II) encoded by (I); (5) method (M5) for identifying a functional human GPR54 receptor protein in a biological sample; (6) method (M6) for identifying human (II); (7) method (M7) for detecting human (II) messenger RNA in a biological sample; (8) bioassay (M8) for identifying a test compound which modulates the activity of human GPR54 receptor protein; (9) method (M9) for allowing progress of a therapeutic regimen designed to alleviate a condition of abnormal expression of a gene product of (I); (10) method (M10) for determining regression, progression or onset of a pathological disorder characterized by a dysfunctional signal transduction; (11) pharmaceutical composition comprising the polypeptide of (2), a carrier, diluent or excipient; (12) method (M12) for preventing or delaying onset of a condition associated with reduced or non-existent levels of human (II) in a subject prone to the disorder; (13) method (M13) for monitoring the efficacy of an agent in correcting an abnormal level of human (II); (14) method (M14) for detecting a binding partner for human (II) in a sample suspected of containing the binding partner; (15) method (M15) of modulating the endogenous signal transducing activity of (II) in a mammal; (16) method (M16) for identifying a ligand that activates a receptor protein; (17) method (M17) for screening for a disorder characterized by expression of a dysfunctional human (II) coded for by a cDNA with a sequence substantially homologous to 1197 bp, given in the specification; (18) method (M18) for identifying agonist or antagonist of human (II); (19) host cell transfected with an isolated nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides that encodes human (II); (20) recombinant non-human cell line which has been

engineered to express a heterologous protein; (21) isolated cell transformed or transfected with a sequence of nucleotides or ribonucleotides under conditions favoring cell surface expression of a functional human or mouse (II); (22) expression vector; (23) isolated nucleic acid molecule comprising a sequence of nucleotides that encodes a mouse (II); (24) isolated nucleic acid molecule comprising a coding region that encodes a splice variant of a mouse (II), where it is encoded by a fully defined sequence of 1191 bp, given in the specification; (25) isolated nucleic acid molecule that encodes a mouse (II) having a fully defined sequence of 396 amino acids, given in the specification; (26) isolated polypeptide encoded by the a sequence that is a splice variant of an isolated nucleic acid molecule that encodes a fully defined sequence of 396 amino acids, given in the specification; (27) isolated cells comprising the nucleic acid molecule of (23); (28) isolated mouse (II) encoded by (I); and (29) antibody that is specific for (II).  
4787

WIDER DISCLOSURE - Also disclosed as new are kits comprising the compositions of the present invention, reagents, probes, primers, labels and a container.

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) and the nucleic acid molecule of (24) is preferably a genomic DNA, mRNA or cDNA. (I) further comprises a nucleotide sequence encoding a polypeptide which has at least 80% identity to a sequence of 398 amino acids, fully defined in the specification, which may include up to Na amino acid alterations over the entire length of the same sequence, where Na is the maximum number of amino acid alterations, and is calculated by Formula A,  $(A) Xa = 398$  amino acids, and  $Y = 0.80$ , where any non-integer product of Xa and Y is rounded down to the nearest integer prior to subtracting the product from Xa. The nucleic acid molecule of (23) that encodes a mouse (II) further comprises: (a) fully defined sequence of 1191 bp, given in the specification; (b) sequence that hybridizes under conditions of high stringency to the complement of the sequence in (a), and if it is DNA, a full complement or an RNA, is identical to mRNA native to a human cell; or (c) degenerate sequence of (a) or (b). Preferred Methods: (M5) comprises introducing (I) into a suitable host cell that expresses a functional (II), and assaying for second messenger activity in the cells. (M6) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The sample in the (M8) is a biological tissue. The probe in (M10) is an antibody, where it is labeled with a radioactive label or enzyme. (M7) comprises contacting all or part of a fully defined sequence of 1197 bp, given in the specification, with the sample under conditions allowing a complex to form between the nucleic acid sequence and the mRNA, detecting the complexes, and determining the level of mRNA. The (M8) comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with DNA encoding human (II) in the absence or presence of the test compound, thereby obtaining a first and second measurement, respectively; and (b) comparing the first and second measurements and identifying those compounds that result in a difference between the two measurements as a test compound that modulates the activity of human (II), where the eukaryotic cells express a functional human parathyroid hormone-2 receptor protein. The (M9) comprises: (a) assaying a sample from a subject to determine the level of a polypeptide encoded by a fully defined sequence of 1197 bp, or a polypeptide with a fully defined sequence of 398 amino acids, both given in the specification, at a first time point; (b) assaying level of the parameter in (a) at a second time point; and (c) comparing the level at the second time point to the level determined in (a) as a determination of effect of the therapeutic regimen. (M10) comprises incubating a sample with a complimentary nucleic acid hybridization probe with a sequence substantially homologous to 1197 bp, given in the specification, or with a detectable probe that is specific for the gene product of (I) under conditions favoring formation



of a probe/gene product complex, and determining binding between the probe and any complimentary mRNA or a formation of a probe/gene product complex that may be present as determinative of the regression, progression or onset of the pathological disorder. (M12) comprises administering (II) to the subject to prevent or delay onset of the condition. (M13) comprises administering the agent and determining a level of (II) following its administration, where a change in the level towards a normal level is indicative of the efficacy of the agent. (M14) comprises contacting with human (II) under conditions favoring binding of the receptor to the binding partner, and determining the presence of the binding partner in the sample by detecting binding of the receptor to the binding partner. (M15) comprises administering the binding partner detected in M(14). (M16) comprises: (a) introducing (II) into receptor protein-deficient cells or contacting endogenous-receptor protein-deficient host cells with candidate ligand, where the cells contain a reporter gene functionally linked to a hormone response element responsive to and induces expression of the reporter gene; (b) challenging the cells with candidate ligand which can potentially bind with the ligand-binding domain of the receptor protein; and (c) monitoring induction of the reporter gene, thereby identifying ligand that activate the receptor protein. (M17) comprises contacting a sample from a subject believed to suffer from the disorder with an antibody specific for an expression product of the same sequence, or contacting a cDNA or mRNA containing sample with a nucleic acid hybridization probe which hybridizes to a cDNA molecule comprising a fully defined sequence of 1197 bp, given in the specification, and determining binding between the antibody and the expression production as an indication of possible presence of the disorder in the subject. (M18) comprises contacting a cell expression on the surface the receptor protein, where the protein is associated with a second component capable of providing a detectable signal in response to the binding of a compound to the receptor, with a compound to be screened under conditions favoring binding of the compound to the receptor protein, and determining whether the compound binds to and activates or inhibits the receptor protein by measuring the level of a signal generated from the interaction of the compound with the receptor protein. Preferred Cell Line: The cell line further comprises a host cell transformed or transfected with a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides that encodes human (II). Preferred Isolated Cells: The isolated cells of (3) and (27) are bacterial cells, mammalian cells or amphibian oocytes, and the molecule is heterologous to the cells. Preferred Vector: The vector of (24) comprises (I) operably linked to a regulatory nucleotide sequence the controls expression of the nucleic acid molecule in a host cell. Preferred Antibody: The antibody of (29) is a monoclonal antibody. Preparation: (I) was prepared by standard recombinant techniques.

ACTIVITY - Antidiabetic; Analgesic, Vasotropic; Antimigraine; Antidepressant; Nootropic; Neuroprotective; Tranquilizer. Test details are described but no results are given.

MECHANISM OF ACTION - Gene Therapy; G-Protein-Agonist; G-Protein-Antagonist. No supporting data provided.

USE - The polypeptide is useful for preventing and/or treating abnormal conditions associated to both an excess of and insufficient amounts of human GPR54 activity, such as eating disorders, diabetes, pain, migraine, anxiety, depression, ischemia, Alzheimer's disease and reproductive and sleep disorders. also The polypeptide can be used to identify compounds which modulate its activity, and to follow progress of a therapeutic regime. The polynucleotide can be used to screen for a disorder or to determine regression, progression or onset of such a disorder, characterized by expression of a dysfunctional human GPR54 receptor protein (all claimed).

ADMINISTRATION - Dosage range of the compositions are from 0.1-100 mug/kg body weight, and the routes of administration include intravenous, subcutaneous, intramuscular, intraperitoneal, transmucosal, transdermal,



oral and topical.

EXAMPLE - A piece of human genomic DNA encoding human G protein-coupled receptor (GPR54) protein was utilized to generate a fragment for plasmid subcloning using specific oligonucleotide primers. First-round PCR was carried out using the primers pair HGPR54.F5 and HGPR54.R7 with the DNA polymerase Taq Gold and the DNA template Marathon-ready human hypothalamus cDNA in the presence of 5% DMSO for 40 cycles. The resulting PCR product was then used as a template for a second-round PCR under the same conditions except only 35 cycles were performed. The resulting (approximately 1300 bp) PCR product from this nested PCR was purified and cloned in to the vector pCR3.1. Clones containing full-length GPR54 were thus identified. (125 pages)

L15 ANSWER 12 OF 33 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002268325 EMBASE  
TITLE: Transcript expression of the tuberoinfundibular peptide (TIP)39/PTH2 receptor system and non-PTH1 receptor-mediated tonic effects of TIP39 and other PTH2 receptor ligands in renal vessels.  
AUTHOR: Eichinger A.; Fiaschi-Taesch N.; Massfelder T.; Fritsch S.; Barthelmebs M.; Helwig J.-J.  
CORPORATE SOURCE: Dr. J.-J. Helwig, Pharmacol./Physiol. Renovasculaires, E. M. I. N. S. Rec. M.-U. L. Pasteur, Batiment 4, 11 rue Humann, F67085 Strasbourg Cedex, France.  
SOURCE: jean-jacques.helwig@pharmaco-ulp.u-strasbg.fr  
Endocrinology, (2002) Vol. 143, No. 8, pp. 3036-3043. .  
Refs: 43  
ISSN: 0013-7227 CODEN: ENDOAO  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 002 Physiology  
003 Endocrinology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 8 Aug 2002  
Last Updated on STN: 8 Aug 2002

AB Although lower than in brain, the type 2 PTH receptor (PTH2-R) has been shown to be expressed throughout the cardiovascular system. Tuberoinfundibular peptide (TIP) purified from brain is thought to be the endogenous selective ligand of the PTH2-R. In the present studies, TIP and PTH2-R mRNA expressions were evidenced by RT-PCR in rat intrarenal arteries as well as in renovascular smooth muscle cells cultured from these arteries. In the isolated perfused rat kidney (IPK), peptides known to bind to both PTH1- and PTH2-Rs, such as rat PTH (1-34) and the hybrid PTH/PTHrP peptide, [Ile(5), Trp(23)]PTHrP (1-36), failed to exhibit improved vasodilatory effect, compared with human PTHrP (1-36), which binds only to the PTH1-R. Thus, a non-PTH1-R seemed not to be involved in the vasodilatory effects of these peptides. On the other hand, TIP exhibited complex vasoactivity, constricting the IPK at 10 nM and dilating the IPK at 1, 100, and 1000 nM. Moreover, [p-benzoyl-L-Phe(4),Ile(5),Trp(23)]PTHrP (1-36), initially described as a selective PTH2-R antagonist, also displayed a strong vasodilatory effect and therefore could not be used to check that TIP-induced vasoactivity was mediated by the PTH2-R. However, both [p-benzoyl-L-Phe(4),Ile(5),Trp(23)]PTHrP (1-36) and TIP displayed similar or even enhanced vasodilation in IPK in which PTH1-R-induced vasodilation was fully desensitized by sustained exposure to human PTHrP (1-36). Importantly, in IPK desensitized to the vasodilatory action of PTHrP (1-36), the hybrid PTH/PTHrP peptide and rat PTH (1-34), whose vasodilatory responses appeared exclusively PTH1-R dependent in naive IPK, produced a new and strong vasodilation. In conclusion, TIP and PTH2-R mRNAs are expressed in renal vessels and TIP appears as a new vasoactive peptide. Whether TIP interacts with PTH2-R could not be shown. However,

these studies reveal the ability of TIP, as well as of other peptides known to bind to the PTH2-R, to dilate renal vessels in a PTH1-R-independent manner. Moreover, results obtained in IPK desensitized to the vasodilatory action of PTHrP (1-36) strongly suggest that TIP, along with PTHrP, might be coordinately involved in the regulation of renal hemodynamics.

L15 ANSWER 13 OF 33 LIFESCI COPYRIGHT 2006 CSA on STN  
ACCESSION NUMBER: 2003:77906 LIFESCI  
TITLE: Expression and Distribution of Tuberoinfundibular Peptide of 39 Residues in the Rat Central Nervous System  
AUTHOR: Dobolyi, A.; Palkovits, M.; Usdin, T.B.  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, 36 Convent Dr., Bethesda, MD 20892-4094, USA; E-mail: usdin@codon.nih.gov  
SOURCE: Journal of Comparative Neurology [J. Comp. Neurol.], (20021209) vol. 455, no. 4, pp. 547-566. ISSN: 0021-9967.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: N3  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Tuberoinfundibular peptide of 39 residues (TIP39) has been recently purified and identified as a selective ligand for the parathyroid hormone 2 receptor. As a next step toward understanding its functions, we report the expression and distribution of TIP39 in the rat central nervous system. In situ hybridization histochemistry and immunocytochemistry revealed TIP39-containing cell bodies in three distinct areas. The major one comprises the subparafascicular area posterior through the intralaminar nucleus of the thalamus; a second is the medial paralemniscal nucleus at the pontomesencephalic junction; and a third is in the dorsal and dorsolateral hypothalamic areas, which contained a few, scattered cell bodies. We found, in contrast to the highly restricted localization of TIP39-containing cell bodies, a much more widespread localization of TIP39-containing fibers. The highest density of fibers was observed in limbic areas such as the septum, the amygdala, and the bed nucleus of the stria terminalis; in areas involved in endocrine regulation, such as the hypothalamic dorsomedial, paraventricular, periventricular, and arcuate nuclei; in auditory areas, such as the ectothalamic and temporal cortices, inferior colliculus, medial geniculate body, and some of the nuclei of the superior olivary complex; and in the dorsolateral funiculus of the spinal cord. The localization of TIP39-containing nuclei and fibers provides an anatomical basis for previously demonstrated endocrine and nociceptive effects of TIP39 and suggests additional functions for TIP39, one apparent candidate being the regulation of auditory information processing.

L15 ANSWER 14 OF 33 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
ACCESSION NUMBER: 2001:246727 BIOSIS  
DOCUMENT NUMBER: PREV200100246727  
TITLE: Parathyroid hormone 2  
-receptor is expressed on human leucocytes and down-regulated in hyperparathyroidism.  
AUTHOR(S): Kosch, M.; Hausberg, M.; Eue, I. [Reprint author]; Usdin, T.; Seeliger, S. [Reprint author]  
CORPORATE SOURCE: Institut fuer Physiologie, Robert-Koch-Str. 27a, D-48149, Muenster, Germany  
SOURCE: Pfluegers Archiv European Journal of Physiology, (2001) Vol. 441, No. 6 Supplement, pp. R156. print. Meeting Info.: Joint Congress of the Scandinavian and the German Physiological Societies. Berlin, Germany. March 10-13, 2001. CODEN: PFLABK. ISSN: 0031-6768.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
Conference; (Meeting Poster)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 23 May 2001  
Last Updated on STN: 19 Feb 2002

L15 ANSWER 15 OF 33 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2001:486661 BIOSIS  
DOCUMENT NUMBER: PREV200100486661  
TITLE: Distribution of mRNA encoding the peptide neuromodulator  
candidate TIP39 in the nervous system.  
AUTHOR(S): Dobolyi, A. [Reprint author]; Palkovits, M. [Reprint  
author]; Mezey, E.; Usdin, T. B. [Reprint author]  
CORPORATE SOURCE: Lab. Genetics, NIMH, NIH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1,  
pp. 70. print.  
Meeting Info.: 31st Annual Meeting of the Society for  
Neuroscience. San Diego, California, USA. November 10-15,  
2001.  
ISSN: 0190-5295.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 17 Oct 2001  
Last Updated on STN: 23 Feb 2002

AB We recently purified tuberoinfundibular peptide of 39 residues  
(TIP39) from bovine hypothalamus and demonstrated that it is a potent and  
selective parathyroid hormone 2 (PTH2)  
receptor agonist. Our previous observation that the PTH2 receptor's CNS  
distribution is topographically widespread suggests that TIP39 may be  
involved in a variety of neural functions. As the next step toward  
defining its roles, we have begun to examine TIP39's distribution. We  
identified TIP39's human and mouse genomic sequences and used them to  
predict its mRNA sequence, which we confirmed in rat and mouse by RT-PCR.  
Using RT-PCR as a screen, we found TIP39 mRNA in rat diencephalon,  
brainstem, cerebellum and dorsal root ganglia, but not in cerebral cortex,  
hippocampus and spinal cord. To investigate the distribution of TIP39  
mRNA at the cellular level we performed in situ hybridization  
histochemistry (ISHH) on sections from rat brain and spinal cord using two  
non-overlapping 35S-labelled RNA TIP39 probes. In all 7 animals tested,  
two groups of intensely labeled cells were present: one in the most medial  
part of the central gray at the hypothalamic-midbrain junction; the other  
in the lateral pons bordered by the ventral lateral lemniscal and  
Kolliker-Fuse nuclei. We confirmed these ISHH observations by RT-PCR of  
microdissected tissue pellets. Other areas had much less intense labeling  
and are currently being analyzed. The fine topographical distribution of  
TIP39 neurons will facilitate experimental studies to clarify its  
function.

L15 ANSWER 16 OF 33 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights  
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ACCESSION NUMBER: 2001129864 EMBASE  
TITLE: Evaluating the ligand specificity of zebrafish parathyroid  
hormone (PTH) receptors: Comparison of PTH, PTH-related  
protein, and tuberoinfundibular peptide of 39 residues.  
AUTHOR: Hoare S.R.J.; Rubin D.A.; Juppner H.; Usdin T.B.  
CORPORATE SOURCE: Dr. T.B. Usdin, Unit on Cell Biology, Laboratory of  
Genetics, National Institute of Mental Health, 36 Convent  
Drive, Bethesda, MD 20892-4094, United States.  
usdin@codon.nih.gov  
SOURCE: Endocrinology, (2000) Vol. 141, No. 9, pp. 3080-3086. .  
Refs: 22

ISSN: 0013-7227 CODEN: ENDOAO  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 003 Endocrinology  
030 Pharmacology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 19 Apr 2001  
Last Updated on STN: 19 Apr 2001

AB Homologs of mammalian PTH1 and PTH2 receptors, and a novel PTH3 receptor have been identified in zebrafish (zPTH1, zPTH2, and zPTH3), zPTH1 receptor ligand specificity is similar to that of mammalian PTH1 receptors. The zPTH2 receptor is selective for PTH over PTH-related protein (PTHrP); however, PTH produces only modest cAMP accumulation. A PTH2 receptor-selective peptide, tuberoinfundibular peptide of 39 residues (TIP39), has recently been purified from bovine hypothalamus. The effect of TIP39 has not previously been examined on zebrafish receptors. The zPTH3 receptor was initially described as PTHrP selective based on comparison with the effects of human PTH. We have now examined the ligand specificity of the zebrafish PTH-recognizing receptors expressed in COS-7 cells using a wide range of ligands. TIP39 is a potent agonist for stimulation of cAMP accumulation at two putative splice variants of the zPTH2 receptor (EC(50), 2.6 and 5.2 nM); in comparison, PTH is a partial agonist [maximal effect (E(max)) of PTH peptides ranges from 28-49% of the TIP39 E(max)]. As TIP39 is much more efficacious than any known PTH-like peptide, a homolog of TIP39 may be the zPTH2 receptor's endogenous ligand. At the zPTH3 receptor, rat PTH-(1-34) and rat PTH-(1-84) (EC(50), 0.22 and 0.45 nM) are more potent than PTHrP (EC(50), 1.5 nM), and rPTH-(1-34) binds with high affinity (3.2 nM). PTH has not been isolated from fish. PTHrP-like peptides, which have been identified in fish, may be the natural ligands for zPTH1 and zPTH3 receptors.

L15 ANSWER 17 OF 33 MEDLINE on STN DUPLICATE 7  
ACCESSION NUMBER: 2001077468 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11098126  
TITLE: Distribution of parathyroid hormone-  
2 receptor-like immunoreactivity and messenger RNA  
in the rat nervous system.  
AUTHOR: Wang T; Palkovits M; Rusnak M; Mezey E; Usdin T B  
CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National  
Institute of Mental Health, 36 Convent Drive MSC4094,  
Bethesda, MD 20892-4094, USA.  
SOURCE: Neuroscience, (2000) Vol. 100, No. 3, pp. 629-49.  
Journal code: 7605074. ISSN: 0306-4522.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200101  
ENTRY DATE: Entered STN: 22 Mar 2001  
Last Updated on STN: 22 Mar 2001  
Entered Medline: 11 Jan 2001

AB The parathyroid hormone-2 receptor is a member of the secretin family of guanine nucleotide-binding protein-coupled receptors. The human parathyroid hormone-2 receptor is activated by parathyroid hormone and a recently purified hypothalamic polypeptide, tubero-infundibular peptide of 39 residues, while the rat parathyroid hormone-2 receptor is poorly activated by parathyroid hormone and is potently activated by tubero-infundibular peptide of 39 residues. In order to provide a foundation for studies on the physiological role of the parathyroid hormone-2 receptor and



tubero-infundibular peptide of 39 residues, we investigated the cellular distribution of the parathyroid hormone-2 receptor in the rat CNS using both immunohistochemistry and in situ hybridization histochemistry. The receptor is found in discrete groups of neurons in many regions. It is present in scattered small cells throughout the cerebral cortex, in small and medium-sized cells in the striatum, and is quite abundant in the septum and the midline thalamic nuclei. Its expression is high in the hypothalamus, particularly in the periventricular and arcuate nuclei. Fibers and terminals in the external zone of the median eminence, and in the superficial layers of the caudal spinal trigeminal tract and the spinal cord dorsal horn, are strongly and dramatically labeled by a parathyroid hormone-2 receptor-selective antibody. The localization of parathyroid hormone-2 receptor suggests a role in the regulation of pituitary hormone secretion, sensory information processing and homeostatic regulation.

L15 ANSWER 18 OF 33 MEDLINE on STN DUPLICATE 8  
 ACCESSION NUMBER: 2001034296 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 11013069  
 TITLE: New members of the parathyroid hormone/parathyroid hormone receptor family: the parathyroid hormone 2 receptor and tuberoinfundibular peptide of 39 residues.  
 AUTHOR: Usdin T B; Wang T; Hoare S R; Mezey E; Palkovits M  
 CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland 20892-4094, USA..  
 usdin@codon.nih.gov  
 SOURCE: Frontiers in neuroendocrinology, (2000 Oct) Vol. 21, No. 4, pp. 349-83. Ref: 160  
 Journal code: 7513292. ISSN: 0091-3022.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200011  
 ENTRY DATE: Entered STN: 22 Mar 2001  
 Last Updated on STN: 22 Mar 2001  
 Entered Medline: 30 Nov 2000  
 AB The parathyroid hormone (PTH) family currently includes three peptides and three receptors. PTH regulates calcium homeostasis through bone and kidney PTH1 receptors. PTH-related peptide, probably also through PTH1 receptors, regulates skeletal, pancreatic, epidermal, and mammary gland differentiation and bladder and vascular smooth muscle relaxation and has a CNS role that is under investigation. Tuberoinfundibular peptide of 39 residues (TIP39) was recently purified from bovine hypothalamus based on selective PTH2 receptor activation. PTH2 receptor expression is greatest in the CNS, where it is concentrated in limbic, hypothalamic, and sensory areas, especially hypothalamic periventricular neurons, nerve terminals in the median eminence, superficial layers of the spinal cord dorsal horn, and the caudal part of the sensory trigeminal nucleus. It is also present in a number of endocrine cells. Thus TIP39 and PTH2 receptor-influenced functions may range from pituitary and pancreatic hormone release to pain perception. A third PTH-recognizing receptor has been found in zebrafish.  
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L15 ANSWER 19 OF 33 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
 ACCESSION NUMBER: 2001:135269 BIOSIS  
 DOCUMENT NUMBER: PREV200100135269  
 TITLE: Brain administration of tuberoinfundibular peptide of 39 residues inhibits growth hormone secretion.

AUTHOR(S): Wang, T. [Reprint author]; Edwards, G. L.; Lange, G. D.;  
Parlow, A. F.; Usdin, T. B.  
CORPORATE SOURCE: NIMH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No.  
1-2, pp. Abstract No.-780.10. print.  
Meeting Info.: 30th Annual Meeting of the Society of  
Neuroscience. New Orleans, LA, USA. November 04-09, 2000.  
Society for Neuroscience.  
ISSN: 0190-5295.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 14 Mar 2001  
Last Updated on STN: 15 Feb 2002

AB We recently purified TIP39, a previously undescribed peptide  
from bovine hypothalamus. It is a potent and selective activator of both  
the rat and human parathyroid hormone 2  
(PTH2) receptors and may be the PTH2 receptor's normal endogenous ligand.  
In the rat CNS the PTH2 receptor is highly expressed in the external layer  
of the median eminence, arcuate nucleus, and somatostatin-containing  
neurons in the hypothalamic periventricular nucleus. This localization  
suggests a role for TIP39 and the PTH2 receptor in regulation of anterior  
pituitary hormone secretion. We have now examined the hypothesis that  
TIP39 is involved in the regulation of growth hormone (GH) secretion by  
investigating the effect of intracerebroventricular (ICV) administration  
of TIP39. Ten male rats were surgically prepared with a guide cannula  
into the lateral ventricle and a catheter into the jugular vein. Each rat  
received ICV administration of 10 ug TIP39 or 10 ul vehicle. Blood  
samples were taken from the jugular vein for GH and prolactin measurement  
every 15 min for three hours. TIP39 caused a significant suppression of  
GH secretion, in terms of the total area under the curve, peak area and  
peak amplitude, as compared to vehicle injection. TIP39 had no effect on  
plasma prolactin levels. These data demonstrate that ICV administration  
of TIP39 exerts a specific inhibitory action on spontaneous GH secretion.  
TIP39 may be an important negative regulator of GH neuroendocrine axis.

L15 ANSWER 20 OF 33 MEDLINE on STN DUPLICATE 9  
ACCESSION NUMBER: 1999427840 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10499494  
TITLE: Comparison of rat and human parathyroid  
hormone 2 (PTH2) receptor activation: PTH  
is a low potency partial agonist at the rat PTH2 receptor.  
AUTHOR: Hoare S R; Bonner T I; Usdin T B  
CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National  
Institute of Mental Health, Bethesda, Maryland 20892-4094,  
USA.  
SOURCE: Endocrinology, (1999 Oct) Vol. 140, No. 10, pp. 4419-25.  
Journal code: 0375040. ISSN: 0013-7227.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 199910  
ENTRY DATE: Entered STN: 26 Oct 1999  
Last Updated on STN: 26 Oct 1999  
Entered Medline: 12 Oct 1999

AB The human PTH2 receptor, expressed in tissue culture cells, is selectively  
activated by PTH. Detailed investigation of its anatomical and cellular  
distribution has been performed in the rat. It is expressed by neurons in  
a number of brain nuclei; by endocrine cells that include pancreatic islet  
somatostatin cells, thyroid parafollicular cells, and peptide secreting  
cells in the gastrointestinal tract; and by cells in the vasculature and  
heart. The physiological role of the PTH2 receptor expressed by these  
cells remains to be determined. All pharmacological studies performed to

date have used the human receptor. We have now isolated a complementary DNA including the entire coding sequence of the rat PTH2 receptor and compared its pharmacological profile with that of the human PTH2 receptor when each is expressed in COS-7 cells. PTH-based peptides, including rat PTH(1-84), rat PTH(1-34), and human PTH(1-34), have low potency at the rat PTH2 receptor for stimulation of adenylyl cyclase (EC50 = 19-140 nM). When compared with the effect of a bovine hypothalamic extract, PTH-based peptides are partial agonists at the rat PTH2 receptor. This suggests that PTH is unlikely to be a physiologically important endogenous ligand for the PTH2 receptor. A peptide homologous to an activity detected in a bovine hypothalamic extract is a good candidate for the endogenous PTH2 receptor ligand.

L15 ANSWER 21 OF 33 MEDLINE on STN DUPLICATE 10  
 ACCESSION NUMBER: 97156632 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 9003022  
 TITLE: Evidence for a parathyroid hormone-  
 2 receptor selective ligand in the hypothalamus.  
 AUTHOR: Usdin T B  
 CORPORATE SOURCE: National Institute of Mental Health, Bethesda MD 20892,  
 USA.. usdin@codon.nih.gov  
 SOURCE: Endocrinology, (1997 Feb) Vol. 138, No. 2, pp. 831-4.  
 Journal code: 0375040. ISSN: 0013-7227.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
 ENTRY MONTH: 199702  
 ENTRY DATE: Entered STN: 5 Mar 1997  
 Last Updated on STN: 5 Mar 1997  
 Entered Medline: 18 Feb 1997

AB The PTH2 receptor is expressed in several brain nuclei but we have been unable to detect mRNA encoding PTH, which is the only known ligand for the PTH2 receptor, in the brain. We now have evidence for a PTH2 receptor selective ligand in an acid-acetone extract made from bovine hypothalamus. The partially purified extract activates the PTH2 receptor more effectively than it activates the PTH/PTHrP receptor, while PTH activates these two receptors at similar concentration. The activity appears immunologically distinct from PTH and its effect is potently antagonized by [D-Trp12]bPTH(7-34). These data provide evidence for a biologically active peptide, which may be related to PTH, and which is a potential new neurotransmitter or hormone.

L15 ANSWER 22 OF 33 MEDLINE on STN DUPLICATE 11  
 ACCESSION NUMBER: 93215928 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8462737  
 TITLE: Characterization of the mineralization process in cultures  
 of rabbit growth plate chondrocytes.  
 AUTHOR: Jikko A; Aoba T; Murakami H; Takano Y; Iwamoto M; Kato Y  
 CORPORATE SOURCE: Department of Radiology, Faculty of Dentistry, Osaka  
 University, Japan.  
 CONTRACT NUMBER: DE08670 (NIDCR)  
 SOURCE: Developmental biology, (1993 Apr) Vol. 156, No. 2, pp.  
 372-80.  
 Journal code: 0372762. ISSN: 0012-1606.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals; Space Life Sciences  
 ENTRY MONTH: 199304  
 ENTRY DATE: Entered STN: 21 May 1993  
 Last Updated on STN: 3 Mar 2000  
 Entered Medline: 30 Apr 1993

AB The present study was undertaken to investigate the mineralization process

in chondrocyte cultures. Chondrocytes were isolated from the growth plate of ribs of 4-week-old rabbits. The nature and properties of mineral crystals precipitated in chondrocyte cultures were compared with those of crystals formed in the hypertrophic zone and bone of rabbit rib growth plates in vivo. The chondrocytes were maintained at high density on type II collagen-coated dishes in Eagle's medium, alpha-modification, with 10% fetal bovine serum and 50 micrograms/ml of ascorbic acid. These cells differentiated into hypertrophic cells 10 days after seeding and produced alkaline phosphatase and 1,25-dihydroxyvitamin D3 receptors on Days 30-70 at levels as high as those in the lower hypertrophic zone in vivo. Mineralization was initiated between Days 20 and 30 and advanced progressively throughout the culture period. However, mineralization was suppressed by the addition of parathyroid hormone ( $2 \times 10^{-8}$  M) or by the presence of fibroblasts. Examination by electron microscopy and Fourier transform infrared (FTIR) spectroscopy verified that mineralized nodules formed in vitro were composed of small apatite crystals. Importantly, FTIR spectral features of the apatite crystals (e.g., the prominent PO<sub>4</sub> bands at 1125 and 1032 cm<sup>-1</sup>) were similar to those of cartilage apatites formed in vivo and differed markedly from those of carbonated bone apatites. These results suggest that growth plate chondrocytes cultured on collagen-coated dishes are an appropriate model for studies on cartilage mineralization.

L15 ANSWER 23 OF 33 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1989:33825 HCAPLUS

DOCUMENT NUMBER: 110:33825

TITLE: Clinical and laboratory evaluation of a two-site immunoradiometric assay for intact parathyroid hormone

AUTHOR(S): Newman, D. J.; Ashby, J. P.

CORPORATE SOURCE: Dep. Clin. Chem., Northwick Park Hosp., Harrow/Middlesex, HA1 3UJ, UK

SOURCE: Annals of Clinical Biochemistry (1988), 25(6), 654-60  
CODEN: ACBOBU; ISSN: 0004-5632

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The anal. performance and clin. utility of a direct immunoradiometric assay (IRMA) for intact 1-84 human parathyroid hormone (PTH) was evaluated. The assay is available com. and utilizes 2 affinity purified region-specific antisera against either 1-34 (radiolabeled antibody) or 39-84 (solid phase antibody) human PTH. High assay sensitivity (detection limit, 1.4 pg 1-84 PTH/mL) permitted the measurement of PTH in all normocalcemic individuals studied. Elevated results were obtained in all patients with histol. proven primary hyperparathyroidism (PHPT) and 34 out of 35 patients with presumptive PHPT. Thirteen out of 24 patients with non-parathyroid hypercalcemia had suppressed results, but the remainder had concns. within the reference range.

L15 ANSWER 24 OF 33 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1988:486687 HCAPLUS

DOCUMENT NUMBER: 109:86687

TITLE: Induction of ornithine decarboxylase activity in isolated chicken osteoblasts by parathyroid hormone: the role of cAMP and calcium

AUTHOR(S): Lowik, C. W. G. M.; Olthof, A. A.; Van Leeuwen, J. P. T. M.; Van Zeeland, J. K.; Herrmann-Erlee, M. P. M.

CORPORATE SOURCE: Lab. Cell Biol. Histol., Univ. Leiden, Leiden, Neth.

SOURCE: Calcified Tissue International (1988), 43(1), 7-18  
CODEN: CTINDZ; ISSN: 0171-967X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The roles of cAMP and Ca<sup>2+</sup> as mediators in parathyroid hormone (PTH)-induced ornithine decarboxylase (ODC) activity in primary cultures of chicken osteoblasts were examined. The induction of ODC activity by PTH is most likely a receptor-mediated process and cAMP is a mediator.



However, 3 different approaches indicate that cAMP is not the exclusive mediator of PTH-induced ODC activity. First, when the dose-response curve of PTH-induced ODC activity is compared with that of PTH-stimulated cAMP production, the ED50 for cAMP production is about 5 times as high as that for the

induction of ODC activity. Second, 1 mM 9-(tetrahydro-2-furanyl)adenine (SQ 22.536) almost completely inhibited PTH-stimulated cAMP production whereas there was only a small inhibitory effect on PTH-induced ODC activity. Third, some PTH fragments unable to stimulate cAMP production were still able to induce ODC activity. Apart from cAMP, an addnl. messenger, most likely  $\text{Ca}^{2+}$ , must be present. Evidence for this concept are the observations that substances affecting extracellular and intracellular  $\text{Ca}^{2+}$  levels (EGTA, A 23187,  $\text{CoCl}_2$ , verapamil) or antagonizing calmodulin (trifluoroperazin, compound 48/80) also strongly affect PTH-induced ODC activity. These effects could not be explained by a pos. interaction of  $\text{Ca}^{2+}$  with the hormone-stimulated cAMP system as 2 mM EGTA strongly enhanced PTH-stimulated cAMP production but at the same time completely inhibited PTH-induced ODC activity. A similar dissociation between hormone-induced cAMP production and induction of ODC activity was found with the  $\text{Ca}^{2+}$ -ionophore A 23187 ( $10^{-7}\text{M}$ ) which inhibited PTH-stimulated cAMP production but strongly enhanced PTH-induced ODC activity. Intracellular  $\text{Ca}^{2+}$ , and possibly calmodulin, in addition to cAMP, are probably involved in PTH-induced ODC activity in chicken osteoblasts. Most probably  $\text{Ca}^{2+}$  is the initial messenger and cAMP acts in a coordinate pattern as a synarchic messenger making the induction of ODC activity by PTH more sensitive to  $\text{Ca}^{2+}$ . Furthermore, the present findings are in agreement with the concept of the existence of 2 receptors or 2 receptor-sites for PTH on osteoblasts. One receptor is coupled to the production of cAMP and is presumably activated when the 1st 2 amino acids of the  $\text{NH}_2$ -terminus of the hormone are present and the other, suggested to be responsible for the increase in intracellular  $\text{Ca}^{2+}$ , is thought to be activated by a region of the hormone sequence between amino acid 3 and 34. Activation of both receptors by the intact hormone PTH(1-84) or its biol. active fragment PTH(1-34) leads to the maximal induction of ODC activity.

L15 ANSWER 25 OF 33 MEDLINE on STN DUPLICATE 12  
ACCESSION NUMBER: 85258588 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 2991044  
TITLE: Identification of a monoclonal antibody which interacts with the parathyroid hormone receptor-adenylate cyclase system in murine bone.  
AUTHOR: Weinshank R L; Cain C D; Vasquez N P; Luben R A  
SOURCE: Molecular and cellular endocrinology, (1985 Jul) Vol. 41, No. 2-3, pp. 237-46.  
Journal code: 7500844. ISSN: 0303-7207.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; Space Life Sciences  
ENTRY MONTH: 198509  
ENTRY DATE: Entered STN: 20 Mar 1990  
Last Updated on STN: 6 Feb 1998  
Entered Medline: 25 Sep 1985

AB We have produced monoclonal antibodies which bind specifically to mouse bone cells and then selected these monoclonal antibodies for their ability to inhibit parathyroid hormone (PTH) responses in mouse cranial bone treated with the (1-34) amino terminal peptide of bovine PTH [bPTH(1-34)]. One clone, designated 3-6, characterized as an IgM(kappa), significantly inhibited the accumulation of cAMP in response to bPTH(1-34) at concentrations of hormone between  $10^{-9}$  and  $10^{-7}$  M. This antibody was subsequently isolated by gel filtration and shown to bind to intact mouse calvariae, with saturation binding occurring at 3 micrograms/ml IgM. A maximal inhibition of approximately 70% of the cAMP accumulation produced in response to  $2.5 \times 10^{-9}$  M (100 ng/ml) bPTH(1-34)

was obtained with 7 micrograms/ml of the purified 3-6 IgM. At this concentration of 3-6 IgM, the half-maximal dose of PTH for activation of cAMP accumulation was increased from  $5 \times 10^{-9}$  M to  $2 \times 10^{-8}$  M with no reduction in maximal levels of cAMP production. The utility of this antibody as an inhibitor was further tested by its ability to block the binding of an iodinated PTH analogue, 125I-[Nle8, Nle18, Tyr34]-bPTH(1-34) to mouse cranial bone. The 3-6 IgM at a concentration of  $5 \times 10^{-8}$  M inhibited 70% of the specific binding of the 125I-labeled analogue. In the absence of parathyroid hormone,  $2 \times 10^{-8}$  M 3-6 IgM produced a 4-fold increase in cAMP above basal levels, as compared to 40-fold maximal increases observed with PTH, indicating a partial PTH agonist activity of this antibody. When tested for effects on other hormones, 3-6 IgM did not inhibit cAMP accumulation produced in response to salmon calcitonin, epinephrine, prostaglandin E2 or cholera toxin. We propose that the 3-6 monoclonal IgM is specific for the PTH receptor or a component of the PTH receptor-adenylate cyclase system and that this or similar antibodies will serve as useful reagents for future molecular characterization of this receptor.

L15 ANSWER 26 OF 33 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1985:575780 HCAPLUS

DOCUMENT NUMBER: 103:175780

TITLE: Glycoproteins and phosphoproteins secreted by cultured chicken osteoblasts

AUTHOR(S): Lowik, Clemens W. G. M.; Feyen, Jean H. M.; Van Zeeland, J. Karel; Herrmann-Erlee, Maria P. M.

CORPORATE SOURCE: Lab. Cell Biol. Histol., Univ. Leiden, Leiden, 2333 AA, Neth.

SOURCE: International Congress Series (1985), 643 (Curr. Adv. Skeletogenesis), 99-104  
CODEN: EXMDA4; ISSN: 0531-5131

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Osteoblasts, isolated from 18-day-old chick embryos, contd. 4 proteins of relative mol. wts. 40, 67, 170, and >200 kilodaltons (kD) which bound to a Con A affinity column. Pulse incubation of the osteoblast cells with 3H-labeled glucosamine for 8 h showed label uptake by the 170 and >200 kD proteins. Autoradiographs of osteoblasts incubated with 32P revealed the presence of 15 phosphoproteins with a major product of 48 kD being formed after 16 h exposure. In the presence of parathyroid hormone (2 units/mL), 4 of the phosphoproteins formed after incubation with 32P were not labeled and phosphorylation of a 35 kD protein was stimulated. Thus, chicken osteoblasts are able to synthesize and secrete glycoproteins and phosphoproteins and parathyroid hormone has a regulatory role in these processes.

L15 ANSWER 27 OF 33 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1984:527910 HCAPLUS

DOCUMENT NUMBER: 101:127910

TITLE: Fetal bovine bone cells synthesize bone-specific matrix proteins

AUTHOR(S): Whitson, S. William; Harrison, Wilbur; Dunlap, Mary K.; Bowers, Daniel E., Jr.; Fisher, Larry W.; Robey, Pamela Gehron; Termine, John D.

CORPORATE SOURCE: Sch. Dent. Med., South. Illinois Univ., Alton, IL, 62002, USA

SOURCE: Journal of Cell Biology (1984), 99(2), 607-14  
CODEN: JCLBA3; ISSN: 0021-9525

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Cells were isolated from both calvaria and the outer cortexes of long bones from 3-5 mo-old bovine fetuses. The cells were identified as functional osteoblasts by indirect immunofluorescence using antibodies

against 3 bone-specific, noncollagenous matrix proteins (osteonectin, the bone proteoglycan, and the bone sialoprotein) and against type I collagen. In sep. expts., confluent cultures of the cells were radiolabeled and shown to synthesize and secrete osteonectin, the bone proteoglycan and the bone sialoprotein by immunopptn. and fluorog. of SDS polyacrylamide gels. Anal. of the radiolabeled collagens synthesized by the cultures showed that they produced predominantly (.apprx.94%) type I collagen, with small amts. of types III and V collagens. In agreement with previous investigators who have employed the rodent bone cell system, it was confirmed in bovine bone cells that (1) there was a typical cAMP response to parathyroid hormone, (2) freshly isolated cells possessed high levels of alkaline phosphatase, which diminished during culture but returned to normal levels in mineralizing cultures, and (3) cells grown in the presence of ascorbic acid and  $\beta$ -glycerophosphate rapidly produced and mineralized an extracellular matrix containing largely type I collagen. Thus, antibodies directed against bone-specific, noncollagenous proteins can be used to clearly identify bone cells in vitro.

L15 ANSWER 28 OF 33 MEDLINE on STN  
 ACCESSION NUMBER: 84278023 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 6465324  
 TITLE: Ontogenesis of hormone-dependent adenylate cyclase in isolated rat nephron segments.  
 AUTHOR: Imbert-Teboul M; Chabardes D; Clique A; Montegut M; Morel F  
 SOURCE: The American journal of physiology, (1984 Aug) Vol. 247, No. 2 Pt 2, pp. F316-25.  
 Journal code: 0370511. ISSN: 0002-9513.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 198409  
 ENTRY DATE: Entered STN: 20 Mar 1990  
 Last Updated on STN: 6 Feb 1998  
 Entered Medline: 17 Sep 1984

AB Ontogenesis of hormone-dependent adenylate cyclase (AC) was investigated in rat kidney by single tubule microassay between two days postnatal and adulthood. This approach allowed us to analyze the kinetics of vasopressin-sensitive AC maturation in its tubular target sites, namely the thick ascending limb and collecting tubule. It was also possible to compare in a single segment--the thick ascending limb--the kinetics of AC ontogenesis for three hormones-- vasopressin, calcitonin, and parathyroid hormone. The results show that 1) 2 days after birth AC is still poorly responsive to vasopressin, especially in the thick ascending limb. By contrast, this segment exhibits marked AC responses to calcitonin and parathyroid hormone. 2) For a given hormone, the kinetics of AC ontogenesis depends on the segment in which the receptor-enzyme complex is located. 3) For a given segment, the pattern of AC maturation is specific for each hormone. These data indicate that the process of tubular AC maturation cannot be accounted for simply by an increase in basolateral membrane area or by the synthesis of new catalytic units. More specific mechanisms must be involved that regulate independently the synthesis of each kind of hormone receptor and/or its coupling to cyclase.

L15 ANSWER 29 OF 33 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 13  
 ACCESSION NUMBER: 1985:232166 BIOSIS  
 DOCUMENT NUMBER: PREV198579012162; BA79:12162  
 TITLE: ROLE OF CATIONS IN THE CYCLIC AMP RESPONSE TO PARATHYROID HORMONE 2. STUDIES IN ISOLATED BONE CELLS.  
 AUTHOR(S): BOONEKAMP P M [Reprint author]

CORPORATE SOURCE: LAB CELL BIOL HISTOL, UNIV LEIDEN, RIJNSBURGERWEG 10, 2333  
AA LEIDEN, NETH  
SOURCE: Proceedings of the Koninklijke Nederlandse Akademie van  
Wetenschappen Series B Palaeontology Geology Physics  
Chemistry Anthropology, (1984) Vol. 87, No. 2, pp. 237-256.  
CODEN: PKNBE3. ISSN: 0920-2250.

DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH

AB The influence of cations was examined on the cAMP metabolism in  
osteoblast-like cells, isolated from newborn mouse calvaria.  
The cAMP response to PTH could be only effectively regulated by  
extracellular Ca, when extracellular Na was absent. Under these  
conditions the effect of extracellular Ca was dependent on the membrane  
potential. Variations of the membrane potential, at 37° C and in  
the cold, and experiments with a phosphodiesterase inhibitor, indicate  
that a maximal responsiveness of adenylate cyclase to PTH, depends on an  
optimal intracellular Ca concentration, that may be regulated by  
extracellular Na. Studies using K-free media or Na-free media in  
combination with the monovalent cationophore monensin and the antibiotic  
oligomycin reveal that intracellular Na directly, or via mobilization of  
Ca from intracellular stores, may regulate the cAMP response to PTH.

L15 ANSWER 30 OF 33 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights  
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ACCESSION NUMBER: 84194920 EMBASE  
DOCUMENT NUMBER: 1984194920  
TITLE: Ontogenesis of hormone-dependent adenylate cyclase in  
isolated rat nephron segments.  
AUTHOR: Imbert-Teboul M.; Chabardes D.; Clique A.; et al.  
CORPORATE SOURCE: Laboratoire de Physiologie Cellulaire, College de France  
11, 75231 Paris Cedex 05, France  
SOURCE: American Journal of Physiology - Renal Fluid and  
Electrolyte Physiology, (1984) Vol. 16, No. 2, pp.  
F316-F325.

CODEN: AJPFDM  
COUNTRY: United States  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 037 Drug Literature Index  
002 Physiology  
021 Developmental Biology and Teratology  
028 Urology and Nephrology  
003 Endocrinology  
029 Clinical Biochemistry

LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Dec 1991  
Last Updated on STN: 10 Dec 1991

AB Ontogenesis of hormone-dependent adenylate cyclase (AC) was investigated  
in rat kidney by single tubule microassay between two days postnatal and  
adulthood. This approach allowed us to analyze the kinetics of  
vasopressin-sensitive AC maturation in its tubular target sites, namely  
the thick ascending limb and collecting tubule. It was also possible to  
compare in a single segment - the thick ascending limb - the kinetics of  
AC ontogenesis for three hormones - vasopressin, calcitonin, and  
parathyroid hormone. The results show that 1) 2 days after birth AC is  
still poorly responsive to vasopressin, especially in the thick ascending  
limb. By contrast, this segment exhibits marked AC responses to  
calcitonin and parathyroid hormone. 2) For a  
given hormone, the kinetics of AC ontogenesis depends on the segment in  
which the receptor-enzyme complex is located. 3) For a given segment, the  
pattern of AC maturation is specific for each hormone. These data  
indicate that the process of tubular AC maturation cannot be accounted for  
simply by an increase in basolateral membrane area or by the synthesis of  
new catalytic units. More specific mechanisms must be involved that



regulate independently the synthesis of each kind of hormone receptor and/or its coupling to cyclase.

L15 ANSWER 31 OF 33 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1979:518888 HCAPLUS

DOCUMENT NUMBER: 91:118888

TITLE: Identification and cell-free translation of mRNA coding for a precursor of parathyroid secretory protein

AUTHOR(S): Majzoub, Joseph A.; Kronenberg, Henry M.; Potts, John T., Jr.; Rich, Alexander; Habener, Joel F.

CORPORATE SOURCE: Howard Hughes Med. Inst., Harvard Med. Sch., Boston, MA, 02114, USA

SOURCE: Journal of Biological Chemistry (1979), 254(15), 7449-55

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Polyadenylated RNA from bovine parathyroid glands was isolated by d.-gradient sedimentation, translated in heterologous cell-free systems, and analyzed electrophoretically on formamide-polyacrylamide gels. The mRNA coding for parathyroid secretory protein was larger than mRNA for preproparathyroid hormone (20 S vs. 7 S) and had an apparent mol. weight of 740,000. Chromatog. analyses of tryptic peptides prepared from the translation product, when compared with peptides prepared from the cellular product, revealed identity of all but 1 peptide; this peptide was present in the translation product but not in the cellular form of parathyroid secretory protein. Anal. of the translation products of the 20 S mRNA on high resolution polyacrylamide gels revealed microheterogeneity; a single major and 3 minor proteins were found that were of similar size and isoelec. point, and all 4 of the proteins were specifically immunopptd. by antisera to parathyroid secretory protein. Protein sequencing of the N-terminal region of the major translation product indicated that it consisted of the sequence of the cellular protein with an N-terminal extension of 18 amino acids. Thus, (1) parathyroid secretory protein is distinct from and is not a precursor of parathyroid hormone; (2) the major parathyroid secretory protein-related translation product, termed pre-parathyroid secretory protein, is a larger precursor of parathyroid secretory protein, and (3) the 4 parathyroid secretory protein-related translation products are structurally closely related to one another.

L15 ANSWER 32 OF 33 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 79132323 EMBASE

DOCUMENT NUMBER: 1979132323

TITLE: C.E.P. - The calcium elevating peptide from the pancreas.

AUTHOR: Kaplan E.L.; Tager H.S.; Klementsich P.; Lagocki R.

CORPORATE SOURCE: United States

SOURCE: Scandinavian Journal of Gastroenterology, (1978) Vol. 13, No. SUPPL. 49, pp. 96. .

CODEN: SJGRA4

COUNTRY: Norway

DOCUMENT TYPE: Journal

FILE SEGMENT: 037 Drug Literature Index

048 Gastroenterology

003 Endocrinology

LANGUAGE: English

AB We have previously demonstrated several years ago that commercial regular insulin (Lilly) produced hypercalcemia when injected into Holtzman rats. The administration of highly purified bovine and porcine insulins, on the other hand, did not result in any changes in serum calcium (Ca) concentration. None of the following G.I. foregut peptides or amines reproduced this effect: gastrin, histamine, glucagon and

cholecystokinin resulted in hypocalcemia, while VIP, GIP, secretin and pancreatic polypeptide did not change the serum Ca in our bioassay animals. Since then, we have extracted and partially purified this calcium-elevating peptide (C.E.P.) from the pig, cow, rabbit and chicken pancreas, utilizing gel filtration and ion-exchange chromatography. Fractions which contain no immunoreactive insulin or parathyroid hormone (2 separate N-terminal assays) result in a mean serum calcium elevation of 0.7mg% or greater at 60 to 90 minutes after a single injection. Sustained hypercalcemia for at least 5 hours has been obtained by injecting rats hourly for four hours with C.E.P. Hypercalcemia occurs in rats following injection of C.E.P. despite thyroparathyroidectomy (acute and 18 hours previously), nephrectomy, adrenalectomy or bowel resection. Fractions containing as little as 2µg protein have produced hypercalcemia in the rat. C.E.P. is a new hypercalcemic peptide which is found in both mammalian and avian pancreas. It appears to be approximately the size of insulin. Its mode of action is independent of parathyroid hormone secretion; most likely it acts directly on bone.

L15 ANSWER 33 OF 33 MEDLINE on STN  
 ACCESSION NUMBER: 76234606 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 181194  
 TITLE: Reversible resistance to the renal action of parathyroid hormone in man.  
 AUTHOR: Tomlinson S; Hendy G N; Pemberton D M; O'Riordan J L  
 SOURCE: Clinical science and molecular medicine, (1976 Jul) Vol. 51, No. 1, pp. 59-69.  
 Journal code: 0367540. ISSN: 0301-0538.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 197609  
 ENTRY DATE: Entered STN: 13 Mar 1990  
 Last Updated on STN: 3 Feb 1997  
 Entered Medline: 25 Sep 1976

AB 1. Normal subjects showed a highly reproducible, rapid increase in plasma adenosine 3':5'-cyclic monophosphate (cyclic AMP) after an intravenous injection of 200 MRC units of highly purified bovine parathyroid hormone. 2. No significant increase in plasma cyclic AMP was observed after administration of bovine parathyroid hormone to patients with severe chronic renal failure. 3. Even when renal function was not impaired, some patients with primary hyperparathyroidism, who had high concentrations of endogenous parathyroid hormone, showed resistance to bovine parathyroid hormone and when this was injected intravenously it caused only a small increase in plasma cyclic AMP. This resistance was reversible since there was marked improvement in the response after parathyroidectomy, when endogenous parathyroid hormone concentration had fallen. 4. It was possible to reproduce this resistance to the hormone by intravenous infusion of bovine parathyroid hormone into normal subjects. When the hormone (1000 MRC units) was infused over 2 h, after an initial increase there was a progressive decline in plasma cyclic AMP concentration and a fall in urinary cyclic AMP excretion. The response to a standard test stimulus (200 MRC units of bovine parathyroid hormone given as a rapid intravenous injection) was examined at intervals after 1000 units of bovine parathyroid hormone had been infused. Initially, the response was severely impaired; at 4 h, partial recovery had occurred and, 24 h after the infusion, recovery of the response was complete. The resistance was therefore reversible. Infusion of the amino-terminal peptide, fragment 1-34, gave the same effect as infusion of intact hormone. Region-specific assays for the hormone were used to show that the concentration of immuno-assayable hormone remained high during the infusions. 5. The mechanism of this reversible resistance to parathyroid hormone remains to be elucidated; it seems unlikely that

circulating hormone fragments could account for the prolonged impairment in the responsiveness to the intact hormone. It is possible that alteration in the formation, intracellular degradation or, perhaps, release of cyclic AMP from the cells, is the cause. Changes in the characteristics of the hormone receptor sites might also explain the phenomenon.

=> 'd his

'D IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (=>).

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

L1 2096 S MAPKAP##  
L2 5 S L1 AND (MAPKAP-2 (W) KINASE?)  
L3 4 DUP REM L2 (1 DUPLICATE REMOVED)  
L4 119389 S PARATHYROID (W) HORMONE##  
L5 6 S L1 AND L4  
L6 5 DUP REM L5 (1 DUPLICATE REMOVED)  
L7 221 S PARATHYROID (W) HORMONE-2  
L8 1 S L1 AND L7  
L9 68 S IMMUN? AND L7  
L10 1 S L1 AND L9  
L11 369433 S SKELETAL (W) MUSCLE  
L12 387 S L4 AND L11  
L13 0 S L7 AND L11  
L14 76 S L7 AND (ISOLAT? OR PURIF?)  
L15 33 DUP REM L14 (43 DUPLICATES REMOVED)

=> s l7 and (express? or excret?)

4 FILES SEARCHED...

L16 87 L7 AND (EXPRESS? OR EXCRET?)

=> dup rem l16

PROCESSING COMPLETED FOR L16

L17 44 DUP REM L16 (43 DUPLICATES REMOVED)

=> d 1-44 ibib ab

L17 ANSWER 1 OF 44 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights  
reserved on STN DUPLICATE 1

ACCESSION NUMBER: 2006226158 EMBASE

TITLE: The distribution and neurochemistry of the  
parathyroid hormone 2 receptor  
in the rat hypothalamus.

AUTHOR: Dobolyi A.; Irwin S.; Wang J.; Usdin T.B.

CORPORATE SOURCE: T.B. Usdin, Laboratory of Genetics, National Institute of  
Mental Health, 35 Convent Dr., Bethesda, MD 20892, United  
States. usdint@mail.nih.gov

SOURCE: Neurochemical Research, (2006) Vol. 31, No. 2, pp. 227-236.

Refs: 28

ISSN: 0364-3190 CODEN: NEREDZ

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 001 Anatomy, Anthropology, Embryology and Histology  
003 Endocrinology

008      Neurology and Neurosurgery  
LANGUAGE:      English  
SUMMARY LANGUAGE:      English  
ENTRY DATE:      Entered STN: 30 May 2006  
Last Updated on STN: 30 May 2006

AB    This study reports the distribution of parathyroid hormone 2 receptor (PTH2R)-immunoreactive fibers in the hypothalamus using fluorescent amplification immunocytochemistry. The pattern of immunolabeling is strikingly similar to that of tuberoinfundibular peptide of 39 residues (TIP39), a peptide recently purified from bovine hypothalamus and proposed to be a ligand of the PTH2R based on pharmacological data. To investigate the anatomical basis of suggestions that TIP39 affects the secretion of several hypophysiotropic hormones we performed double-labeling studies and found that only somatostatin fibers contain PTH2R in the median eminence, which suggests that somatostatin release could be directly regulated via the PTH2R. However, several hypothalamic nuclei projecting to the median eminence contain a high density of both TIP39 and PTH2R fibers and terminals. We report here, that the PTH2R terminals also contain vesicular glutamate transporter-2, and suggest that TIP39 terminals are ideally positioned to modulate glutamatergic influences on hypophysiotropic neurons. .COPYRGT. Springer Science+Business Media, Inc. 2006.

L17    ANSWER 2 OF 44    HCAPLUS    COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER:      2004:19799    HCAPLUS  
DOCUMENT NUMBER:      140:88757  
TITLE:      Identification of cartilage disease markers by gene expression profile analysis and use in drug screening  
INVENTOR(S):      Aoki, Mikio; Harada, Hideyuki  
PATENT ASSIGNEE(S):      Sumitomo Pharmaceuticals Co., Ltd., Japan  
SOURCE:      Jpn. Kokai Tokkyo Koho, 197 pp.  
CODEN: JKXXAF  
DOCUMENT TYPE:      Patent  
LANGUAGE:      Japanese  
FAMILY ACC. NUM. COUNT:    1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2004000209	A2	20040108	JP 2003-119152	20030424
PRIORITY APPLN. INFO.:			JP 2002-125914	A 20020426

AB    Nucleotide and protein sequences of cartilage disease markers, probes and primers targeting those sequences, antibodies to those proteins, and their use in screening of compds. modulating the expression of those genes as candidate for therapeutic agents for cartilage diseases, are disclosed. Expression profile anal. in osteoarthritis rat model and human patients identified 5-HT7 gene, EDG2 gene, EDG1 gene, ET (A) gene, GPR88 gene, PTH2R gene, VIP1R gene, CLIC2 gene, SCN2A gene, ATA1 gene, ABCA1 gene, ABCG2 gene, GAT1 gene, PLTP gene, ENT1 gene, NPT3 gene, and IREG1 gene, as markers for cartilage diseases.

L17    ANSWER 3 OF 44      MEDLINE on STN

DUPLICATE 2

ACCESSION NUMBER:    2003001658      MEDLINE  
DOCUMENT NUMBER:    PubMed ID: 12508326  
TITLE:      Expression and distribution of tuberoinfundibular peptide of 39 residues in the rat central nervous system.  
AUTHOR:      Dobolyi Arpad; Palkovits Miklos; Usdin Ted Bjorn  
CORPORATE SOURCE:    Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland 20892-4094, USA.  
SOURCE:      The Journal of comparative neurology, (2003 Jan 20) Vol. 455, No. 4, pp. 547-66.  
Journal code: 0406041. ISSN: 0021-9967.  
PUB. COUNTRY:      United States



DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200303  
ENTRY DATE: Entered STN: 2 Jan 2003  
Last Updated on STN: 20 Mar 2003  
Entered Medline: 19 Mar 2003

AB Tuberoinfundibular peptide of 39 residues (TIP39) has been recently purified and identified as a selective ligand for the parathyroid hormone 2 receptor. As a next step toward understanding its functions, we report the expression and distribution of TIP39 in the rat central nervous system. In situ hybridization histochemistry and immunocytochemistry revealed TIP39-containing cell bodies in three distinct areas. The major one comprises the subparafascicular area posterior through the intralaminar nucleus of the thalamus; a second is the medial paralemniscal nucleus at the pontomesencephalic junction; and a third is in the dorsal and dorsolateral hypothalamic areas, which contained a few, scattered cell bodies. We found, in contrast to the highly restricted localization of TIP39-containing cell bodies, a much more widespread localization of TIP39-containing fibers. The highest density of fibers was observed in limbic areas such as the septum, the amygdala, and the bed nucleus of the stria terminalis; in areas involved in endocrine regulation, such as the hypothalamic dorsomedial, paraventricular, periventricular, and arcuate nuclei; in auditory areas, such as the ectothalamic and temporal cortices, inferior colliculus, medial geniculate body, and some of the nuclei of the superior olivary complex; and in the dorsolateral funiculus of the spinal cord. The localization of TIP39-containing nuclei and fibers provides an anatomical basis for previously demonstrated endocrine and nociceptive effects of TIP39 and suggests additional functions for TIP39, one apparent candidate being the regulation of auditory information processing.

L17 ANSWER 4 OF 44 MEDLINE on STN DUPLICATE 3  
ACCESSION NUMBER: 2003306019 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12834174  
TITLE: The parathyroid hormone-2  
receptor is expressed on human leukocytes and  
down-regulated in hyperparathyroidism.  
AUTHOR: Seeliger S; Hausberg M; Eue I; Usdin T; Rahn K H; Kosch M  
CORPORATE SOURCE: Department of Paediatrics, University Hospital Munster,  
Germany.  
SOURCE: Clinical nephrology, (2003 Jun) Vol. 59, No. 6, pp. 429-35.  
Journal code: 0364441. ISSN: 0301-0430.  
PUB. COUNTRY: Germany: Germany, Federal Republic of  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200310  
ENTRY DATE: Entered STN: 2 Jul 2003  
Last Updated on STN: 4 Oct 2003  
Entered Medline: 3 Oct 2003

AB BACKGROUND: Parathyroid hormone (PTH) has specific effects on function, migration and proliferation of human leukocytes. These effects may contribute to accelerated atherosclerosis and impaired immune response observed in patients with renal insufficiency. Recently, a new G protein-coupled receptor with substantial implications for vascular function--the PTH2 receptor (PTH2-R)--has been identified, however, expression and distribution in humans and a possible regulation has not yet been studied. We therefore investigated the expression of the PTH2 receptor on human leukocytes in healthy subjects and in patients with hyperparathyroidism. METHODS: PTH2 receptor expression was quantified by flow cytometry (FACS) analysis on monocytes, lymphocytes and granulocytes that were isolated from peripheral blood (hypotonic density gradient centrifugation) and by

immunohistochemistry using a specific alpha-PTH2-R antibody produced in rabbit. Results of 22 patients with hyperparathyroidism (12 renal allograft recipients, 10 hemodialysis patients, mean age 43 +/- 8 years) were compared to 22 age and sex-matched healthy controls. RESULTS: Mean relative antigen density of the PTH2 receptor and percentage of positive cells in healthy subjects was 19 +/- 5 and 90 +/- 6% on granulocytes, 5 +/- 2 and 55 +/- 19% on monocytes, and 24 +/- 7 and 21 +/- 7% on lymphocytes. In patients with hyperparathyroidism, mean antigen density was significantly lower on granulocytes and monocytes (17 +/- 4% and 3 +/- 1%,  $p < 0.01$ , respectively). The percentage of positive cells and mean expression on lymphocytes was not significantly different. A significant and inverse correlation was found between plasma PTH concentrations and the mean PTH2 receptor expression on granulocytes ( $r = -0.41$ ,  $p < 0.05$ ). CONCLUSIONS: The PTH2 receptor is expressed on human granulocytes and--to a lesser degree--on monocytes and lymphocytes. In patients with hyperparathyroidism the PTH2 receptor is down-regulated as function of plasma PTH levels.

L17 ANSWER 5 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
 ACCESSION NUMBER: 2003:348718 BIOSIS  
 DOCUMENT NUMBER: PREV200300348718  
 TITLE: PTH2 receptor-mediated inhibitory effect of parathyroid hormone and TIP39 on cell proliferation.  
 AUTHOR(S): Misiano, Paola; Scott, Boyd B.; Scheideler, Mark A.; Garnier, Martine [Reprint Author]  
 CORPORATE SOURCE: Psychiatry Centre of Excellence for Drug Discovery, Department of Biology, Neuropharmacology Res., GlaxoSmithKline S.p.A., Via A. Fleming 4, IT-30135, Verona, Italy  
 Martine.J.Garnier@gsk.com  
 SOURCE: European Journal of Pharmacology, (16 May 2003) Vol. 468, No. 3, pp. 159-166. print.  
 ISSN: 0014-2999 (ISSN print).  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 30 Jul 2003  
 Last Updated on STN: 30 Jul 2003

AB The parathyroid hormone (PTH) has dual mitogenic and inhibitory effects on cell proliferation, depending on the cell type and experimental conditions. PTH can signal via two different receptors, both positively coupled to the adenylyl cyclase/cyclic AMP pathway which can mimic some of the proliferative effects of PTH. We evaluated the role of the type-2 PTH (PTH2) receptor on cell proliferation in clonal human embryonic kidney HEK293 cells stably expressing the human PTH2 receptor. Using a cyclic AMP-responsive gene-reporter, we confirmed that the tuberoinfundibular peptide (TIP39) and various human (h) PTH fragments including hPTH-(1-34) were potent agonists ( $EC_{50}$  in the range of 0.01-0.3 nM) whereas the bovine (b) PTH peptides b(Tyr34)PTH-(7-34) and its tryptophan derivative b(D-Trp12,Tyr34)PTH-(7-34) behaved as antagonists ( $IC_{50}$ =117 and 249 nM, respectively). hPTH-(1-34) produced a dose-dependent inhibition of cell proliferation ( $EC_{50}$ =8.5+-0.4 nM) after 3 days and this effect was fully reversed by the tryptophan derivative antagonist. The same effect was observed with TIP39 which caused a 30% maximal inhibition. These findings reveal that PTH2 receptor activation can inhibit cell proliferation and might explain the dual functionality of PTH on cell proliferation.

L17 ANSWER 6 OF 44 MEDLINE on STN DUPLICATE 4  
 ACCESSION NUMBER: 2003050743 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12559132  
 TITLE: Tuberoinfundibular peptide of 39 residues (TIP39): molecular structure and activity for parathyroid hormone 2 receptor.  
 AUTHOR: Della Penna K; Kinose F; Sun H; Koblan K S; Wang H

CORPORATE SOURCE: Department of Molecular Pharmacology, Merck Research  
Laboratories, West Point, PA 19486, USA.  
SOURCE: Neuropharmacology, (2003 Jan) Vol. 44, No. 1, pp. 141-53.  
Journal code: 0236217. ISSN: 0028-3908.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200304  
ENTRY DATE: Entered STN: 2 Feb 2003  
Last Updated on STN: 1 May 2003  
Entered Medline: 30 Apr 2003

AB The neuropeptide TIP39 was recently purified from bovine hypothalamus based on the ability of the peptide to activate the parathyroid hormone 2 receptor (PTH2R) (Nat. Neurosci. 2 (1999) 941). PTH2R is abundantly expressed in the nervous system, and its expression pattern suggests that it may play a role in modulation of pituitary function and in nociception. Towards understanding the physiological role of TIP39 and PTH2R, we cloned human, mouse and rat TIP39 gene. Our results revealed that: (1) the mature peptide is processed from a precursor; (2) TIP39 peptide is highly conserved among species; and (3) TIP39 from all species activates adenylyl cyclase and elevates intracellular calcium levels through PTH2R. We also defined and compared the structure-activity relationship of TIP39 on both activation of adenylyl cyclase and calcium mobilization pathways through PTH2R, finding common and differential determinants of TIP39 that are required for these pathways. Furthermore, we observed that TIP39 elevates intracellular calcium levels in primary dorsal root ganglion neurons whereas the peptide inactive on PTH2R do not, suggesting that TIP39 may activate these neurons important for nociception in vivo through PTH2R-dependent mechanisms.

L17 ANSWER 7 OF 44 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2003:67346 SCISEARCH  
THE GENUINE ARTICLE: 630ER  
TITLE: Emerging functions for tuberoinfundibular peptide of 39 residues  
AUTHOR: Usdin T B (Reprint); Dobolyi A; Ueda H; Palkovits M  
CORPORATE SOURCE: NIMH, Genet Lab, Bldg 36, Room 3D06, 36 Convent Dr, Bethesda, MD 20892 USA (Reprint); NIMH, Genet Lab, Bethesda, MD 20892 USA; Nagasaki Univ, Sch Pharmaceut Sci, Dept Mol Pharmacol & Neurosci, Nagasaki 8528521, Japan  
COUNTRY OF AUTHOR: USA; Japan  
SOURCE: TRENDS IN ENDOCRINOLOGY AND METABOLISM, (JAN 2003) Vol. 14, No. 1, pp. 14-19.  
ISSN: 1043-2760.  
PUBLISHER: ELSEVIER SCIENCE LONDON, 84 THEOBALDS RD, LONDON WC1X 8RR, ENGLAND.  
DOCUMENT TYPE: General Review; Journal  
LANGUAGE: English  
REFERENCE COUNT: 27  
ENTRY DATE: Entered STN: 24 Jan 2003  
Last Updated on STN: 24 Jan 2003

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Tuberoinfundibular peptide of 39 residues (TIP39), a neuropeptide recently purified from the hypothalamus, appears to be an endogenous ligand for the parathyroid hormone 2 (PTH2) receptor. PTH2 receptors are present in several central nervous system and peripheral areas and are particularly concentrated in the hypothalamus, limbic areas and the outer layers of the spinal cord dorsal horn. TIP39-containing neuronal cell bodies have been identified in the subparafascicular area and the medial paralemniscal nucleus, two brainstem regions that project widely through the entire neuraxis. Treatment of

hypothalamic explants with TIP39, and intraventricular injection of the peptide, suggest that it might stimulate hypothalamic-releasing factor secretion. Injection of TIP39, and sequestration of endogenous TIP39 by intrathecal injection of an antibody to TIP39, have provided evidence that it is involved in some aspects of pain sensitivity. Thus, TIP39 might be a new neuromodulator.

L17 ANSWER 8 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
ACCESSION NUMBER: 2003:151793 BIOSIS  
DOCUMENT NUMBER: PREV200300151793  
TITLE: Parathyroid hormone-related peptide: A jack of all trades  
that masters gastric responses to stress.  
AUTHOR(S): Blackshaw, L. Ashley [Reprint Author]  
CORPORATE SOURCE: Nerve-Gut Research Laboratory, Frome Road, Room 1-216-H  
Hanson Center, Adelaide, SA, 5000, Australia  
ablacksh@mail.rah.sa.gov.au  
SOURCE: Journal of Gastroenterology and Hepatology, (January 2003)  
Vol. 18, No. 1, pp. 1-3. print.  
CODEN: JGHEEO. ISSN: 0815-9319.  
DOCUMENT TYPE: Article  
Editorial  
LANGUAGE: English  
ENTRY DATE: Entered STN: 19 Mar 2003  
Last Updated on STN: 19 Mar 2003

L17 ANSWER 9 OF 44 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-08555 BIOTECHDS  
TITLE: New isolated nucleic acid molecule encoding a human  
mitogen-activated protein kinase activating protein kinase-2  
(MAPKAP-2), useful for treating immune-system related  
disorders, inflammation and arthritis;  
recombinant enzyme protein production and sense and  
antisense sequence for use in gene therapy  
AUTHOR: LOGRASSO P; HAWKINS J; LISNOCK J M  
PATENT ASSIGNEE: MERCK and CO INC  
PATENT INFO: WO 2002090524 14 Nov 2002  
APPLICATION INFO: WO 2002-US5670 25 Feb 2002  
PRIORITY INFO: US 2001-272260 28 Feb 2001; US 2001-272260 28 Feb 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-111970 [10]

AB DERWENT ABSTRACT:

NOVELTY - An isolated nucleic acid molecule (I) comprising a sequence of nucleotides that encode a human mitogen-activated protein kinase activating protein kinase-2 (MAPKAP-2 kinase), and a coding region that encodes a splice variant of a MAPKAP-2 kinase, is new.

DETAILED DESCRIPTION - The nucleotide is selected from sequences that: (a) Encode a human MAPKAP-2 kinase and comprise a sequence of 1191 (S1) or 1203 (S2) bp given in the specification; (b) Encode a human MAPKAP-2 kinase and hybridize under conditions of high stringency to the complement of S1 or S2, and, if it is DNA, is fully complementary, or if its is RNA, is identical to mRNA native to a human cell; (c) Degenerate with the MAPKAP-2 polypeptide and encode sequence of (a) or (b); and (d) Encode a sequence having 396 (S3) or 400 (S4) amino acids given in the specification. INDEPENDENT CLAIMS are also included for the following: (1) A polypeptide (II) comprising (S3), or a variant at least 80% identical to (S3) and differs from (S3) only in one or more amino acid substitutions, additions of terminal amino acid residues and/or deletions of terminal amino acid residues, where the ability to phosphorylate Hsp-27 is not diminished; (2) Host cells (III) transfected or transformed with (I), where the cells are bacterial cells, mammalian cells or amphibian oocytes and the nucleic acid molecule is heterologous to the cells; (3) Detecting (M1) MAPKAP-2 messenger RNA in a biological sample; (4) Identifying (M2) DNA sequences encoding a MAPKAP-2 kinase; (5)



Identifying (M3) MAPKAP-2 kinase in a sample; (6) Bioassay (M4) for identifying a compound or reagent which modulates activity of human MAPKAP-2 kinase; (7) Monitoring (M5) the effectiveness of a treatment with a test compound for MAPKAP-2-mediated disease state; (8) Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase; (9) Screening (M7) test compounds for use as inflammation inhibitors; (10) Monitoring (M8) the efficacy of an agent in correcting an abnormal level of the above polypeptide in a prone subject; (11) Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase; (12) An antibody specific for the gene product of (I); (13) A recombinant non-human cell line that has been engineered to express a heterologous protein, comprising (III); (14) An expression vector comprising (I) operably linked to a regulatory nucleotide sequence that controls the expression of the nucleic acid molecule; (15) Detecting (M10) a binding partner for a MAPKAP-2 kinase in a sample suspected of having the binding partner; (16) Identifying (M11) a compound which modulates the binding or kinase activity of a kinase polypeptide having S2; (17) Modulating (M12) endogenous signal transducing activity of the MAPKAP-2 kinase in a mammal; (18) Identifying (M13) a compound which modulates the binding or kinase activity of a naturally-occurring allelic variant of the polypeptide in (S3); (19) Phosphorylating (M14) a serine-containing substrate; (20) Identifying (M15) a reagent that modulates MAPKAP-2 activity; (21) Identifying (M16) a reagent that modulates MAPKAP-2 synthesis; (22) Identifying (M17) a reagent that modulates MAPKAP-2 expression; (23) Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity; and (24) Kits for detecting MAPKAP-2 or the nucleic acid molecule, comprising: (a) a buffer and a labeled antibody which specifically binds to a MAPKAP-2 kinase having serine, threonine, and tyrosine kinase activity, where the sample to be tested is mixed with the buffer and the antibody; or (b) a buffer and a nucleic acid molecule comprising at least about 20 nucleotides capable of hybridizing to a nucleic acid sequence encoding MAPKAP-2 or its complement under stringent hybridization conditions, and instructions for use.

BIOTECHNOLOGY - Preparation: The nucleic acid was prepared using standard isolation techniques. Preferred Nucleic Acid: The isolated nucleic acid molecule is cDNA. The nucleic acid molecule may also comprise a nucleotide sequence encoding a polypeptide that has at least 80% identity to S3, where the 80% identity defines the amino acid alterations allowed for S3 which are determined by the equation:  $N_a = X_a - (X_a Y)$ ;  $N_a$  = maximum number of amino acid alterations;  $X_a$  = total number of amino acids in S3; and  $Y$  = a value of 0.80 Any non-integer product of  $X_a$  and  $Y$  is rounded down to the nearest integer prior to subtracting the product from  $X_a$ . The nucleic acid molecule has a sequence that is at least 80% identical to a nucleotide sequence encoding the above polypeptide. Preferred Polypeptide: (II) is a human MAPKAP-2 kinase encoded by (I) or a splice variant that encodes a MAPKAP-2 kinase comprising (S3) or by a nucleotide acid molecule comprising a sequence that hybridizes to the complement of (S1). Preferred Method: (M1) comprises introducing (I) into a host cell suspected of expressing a MAPKAP-2 kinase to form a complex, and detecting the presence of the complex. In (M2), identifying DNA sequences encoding a MAPKAP-2 kinase comprises probing a cDNA library or a genomic library with a labeled probe, and recovering from the library those sequences having a significant degree of homology relative to the probe, where the probe comprises (I). (M3) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The bioassay (M4) for identifying a test compound which modulates the activity of a human MAPKAP-2 kinase, comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with the DNA encoding the kinase in the absence of the test compound to obtain a first measurement; (b) measuring

the second messenger activity of the eukaryotic cells in the presence of the test compound to obtain a second measurement; and (c) comparing the first and second measurements and identifying those compounds that result in a difference between the 2 measurements as a test compound that modulates the activity of the MAPKAP-2 kinase, where the eukaryotic cells express a functional human parathyroid hormone -2 polypeptide. In (M5), monitoring comprises: (a) obtaining a pre-administration sample from a subject suspected of having a dysfunctional MAPKAP-2-mediated disease; (b) detecting a level of expression or activity of a MAPKAP-2 kinase-encoding mRNA or genomic DNA in the pre-administration sample to obtain a first measurement; (c) detecting a level of expression or activity of the MAPKAP-2 kinase-encoding mRNA or genomic DNA in a post-administration sample to obtain a second measurement; (d) comparing the level of expression or activity of the kinase in the first and second measurements; and (e) altering the administration of the compound to the subject accordingly. Determining (M6) regression, progression or onset of a disease state manifested by a dysfunctional signal transducing MAPKAP-2 kinase, comprises: (a) contacting a cDNA or mRNA containing sample from a subject suspected of suffering from the disease, with the nucleic acid hybridization probe under conditions favoring binding of the probe to the cDNA or mRNA to form a complex; and (b) detecting the complex as an indication that the subject is at risk of developing the disease state. Alternatively, (M6) comprises contacting a sample from a patient with the disorder with a detectable probe that is specific for the gene product of (I), where formation of the probe/gene product complex indicates regression, progression or onset of the pathological disorder in the patient. The probe is an antibody labeled with a radioactive label or an enzyme. (M7) comprises contacting a test compound with a MAPKAP-2 kinase encoded by (I), and testing the contacted kinase protein for its ability to bind or to phosphorylate Hsp-27, where a test compound that inhibits the binding of the MAPKAP-2 kinase protein to the Hsp-27 is a candidate drug for treating inflammation. In (M8), monitoring comprises administering the agent and determining the level of the polypeptide, where a change in the level of the polypeptide towards a normal level indicates the efficacy of the agent. Identifying (M9) ligand(s) that activate a MAPKAP-2 kinase, comprises: (a) contacting endogenous MAPKAP-2 kinase-deficient host cells with a candidate compound suspected of activating the kinase activity, where the host cells contain a reporter gene functionally linked to a transcriptional control element; and an exogenous gene encoding the kinase, where the transcriptional control element, upon activation, induces expression of the reporter gene(s); (b) monitoring induction of the reporter gene(s); and (c) identifying ligand(s) that activate the polypeptide. (M10) comprises contacting the sample with the MAPKAP-2 under conditions favoring binding of the kinase to the binding partner, and determining the presence of the binding partner in the sample by detecting the binding of the kinase to the binding partner. In (M11), identifying comprises contacting a cell expressing the polypeptide with a test compound under conditions suitable for modulation of the binding or kinase activity of the polypeptide, and detecting the modulation of the activity or the binding of the kinase polypeptide by the test compound. Preferably, the agent inhibits or stimulates MAPKAP-2 activity, or modulates the expression of MAPKAP-2 by modulating transcription of a MAPKAP-2 gene or translation of a MAPKAP-2 mRNA. The agent may be an antibody that specifically binds to the kinase, or a nucleic acid molecule having a sequence that is antisense to the coding strand of the MAPKAP-2 mRNA or gene. (M12) comprises contacting a cell capable of expressing MAPKAP-2 with the compound as in (M11). Modulation of the activity of the polypeptide is detected by direct binding of the test compound to the polypeptide, or by using an assay for MAPKAP-2 kinase activity (based on the phosphorylation of a MAPKAP-2 substrate). Direct binding may be determined by lysing the cell, and performing an immunoprecipitation. In addition, the direct binding may be determined by a yeast 2-hybrid assay.

In (M13), the allelic variant is encoded by the nucleic acid molecule which hybridizes to the complement of a nucleic acid molecule consisting of (S1) in 6 x SSC at 45 degrees C, followed by one or more washes in 0.2 x SSC, 0.1% SDS at 50-65 degrees C, and the method comprises: (a) contacting a cell expressing the allelic variant with a test compound under conditions that modulate the binding or kinase activity of the variant; and (b) detecting modulation of the binding or kinase activity of the allelic variant by the test compound. (M14) comprises: (a) incubating the substrate with a concentration of ATP and an enzyme having at least 84% homology to (S1); and (b) measuring the amount of phosphorylation of the substrate. The method further comprises forming a mixture of the enzyme and a candidate antagonist or agonist of the enzyme, and measuring the effect of the candidate antagonist or agonist on the amount of phosphorylation of the substrate. In (M15), identifying comprises: (a) obtaining a test sample containing the MAPKAP-2 kinase and a reagent; (b) incubating the test sample with MAPKAP-2 substrate and with labeled phosphate under conditions that allow phosphorylation of the substrate; (c) determining the rate of incorporation of labeled phosphate into the substrate, where the rate of incorporation is a measure of MAPKAP-2 activity; and (d) comparing the effect of the reagent on MAPKAP-2 activity relative to a control, where a change in the activity indicates the presence of a reagent capable of modulating MAPKAP-2 activity. The MAPKAP-2 substrate is Hsp-25, Hsp-27 or ALT2. The modulation is inhibition of MAPKAP-2 activity. The reagent is an antisense oligonucleotide, a ribozyme, a tumor necrosis factor or an interleukin-1. (M16) comprises: (a) providing a test sample containing the MAPKAP-2 kinase; (b) incubating the sample in the presence of a reagent; (c) fractionating proteins present in the sample by gel electrophoresis; (d) transferring the proteins onto a membrane; (e) probing the proteins with a labeled antibody specific to the MAPKAP-2 kinase, where the level of the synthesis is determined by the amount of the antibody detected; and (f) comparing the effect of the reagent on MAPKAP-2 synthesis relative to a control, where a change in the synthesis indicates the presence of a reagent that modulates MAPKAP-2 synthesis. (M17) comprises: (a) providing a test sample in which a MAPKAP-2 polynucleotide is expressed; (b) incubating the sample in the presence of a reagent; (c) isolating polyadenylated RNA from the sample; (d) incubating the RNA with a polynucleotide probe specific for MAPKAP-2 kinase; (e) determining the amount of the probe hybridized to the RNA, where a level of expression of MAPKAP-2 is directly related to the amount of MAPKAP-2 probe hybridized to the RNA; and (f) comparing the effect of the reagent on MAPKAP-2 expression relative to a control, where a change in the expression indicates the presence of a reagent that modulates MAPKAP-2 expression. Treating (M18) a subject having a disorder associated with aberrant MAPKAP-2 kinase or nucleic acid expression or activity, comprises administering an agent which is a MAPKAP-2 modulator to the subject. The MAPKAP-2 modulator is a MAPKAP-2 kinase, MAPKAP-2 nucleic acid molecule, a peptide, a peptidomimetic, or other small molecule. The disorder is an immune-related disorder.

ACTIVITY - Immunomodulator; Antiinflammatory; Cytostatic; Antiarthritic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Serine-Threonine-Kinase.

USE - (I) encodes (II) which may be used to treat an immune-related disorder (claimed). (I) is especially useful in regulating signal transduction in a cell, and in diagnosing or treating MAPKAP-2-mediated disorders, e.g. cell proliferative disorders, immune system disorders, inflammation, arthritis. The nucleic acid and the polypeptide may also be used in screening assays, predictive medicine, diagnostic or prognostic assays, chromosome mapping, tissue typing, pharmacogenomics and in monitoring clinical trials.

ADMINISTRATION - Administration is oral, topical or parenteral (e.g. intraarterial, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal). The



dosage is 0.001-50 mg/kg, preferably 0.1-1.0 mg/kg.  
EXAMPLE - No relevant example given. (150 pages)

L17 ANSWER 10 OF 44 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2003-02758 BIOTECHDS

TITLE: New isolated GPR54 polynucleotides and polypeptides, useful  
for preventing and/or treating disorders associated with an  
excess or deficiency of GPR54 protein, such as diabetes,  
pain, anxiety, depression and Alzheimer's disease;  
vector-mediated recombinant protein gene transfer and  
expression in host cell for use in gene therapy

AUTHOR: LIU Q; CLEMENTS M; MCDONALD T P

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002059344 1 Aug 2002

APPLICATION INFO: WO 2001-US48333 14 Dec 2001

PRIORITY INFO: US 2000-256299 18 Dec 2000; US 2000-256299 18 Dec 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-666905 [71]

AB DERWENT ABSTRACT:

NOVELTY - A new isolated nucleic acid molecule (I) comprises a sequence  
of nucleotides that encodes a human G protein-coupled receptor (GPR54).

DETAILED DESCRIPTION - A new isolated nucleic acid molecule (I)  
comprises a sequence of nucleotides that encodes a human G  
protein-coupled receptor (GPR54), where the sequence of nucleotides are  
selected from: (i) a fully defined sequence of 1197 bp, given in the  
specification; (ii) a sequence that hybridizes under conditions of high  
stringency to the complement of the sequence in (i), and if it is DNA, a  
full complement or RNA, is identical to mRNA native to a human cell; or  
(iii) a degenerate sequence of (i) or (ii). INDEPENDENT CLAIMS are also  
included for the following: (1) isolated nucleic acid molecule comprising  
a coding region that encodes a splice variant of human GPR54 receptor,  
where it is encoded by a fully defined sequence of 1197 bp, given in the  
specification; (2) isolated polypeptide encoded by a nucleotide sequence  
that is a splice variant of an isolated nucleic acid molecule that  
encodes a protein with a fully defined sequence of 398 amino acids, given  
in the specification; (3) isolated cells comprising (I); (4) isolated  
human GPR54 receptor protein (II) encoded by (I); (5) method (M5) for  
identifying a functional human GPR54 receptor protein in a biological  
sample; (6) method (M6) for identifying human (II); (7) method (M7) for  
detecting human (II) messenger RNA in a biological sample; (8) bioassay  
(M8) for identifying a test compound which modulates the activity of  
human GPR54 receptor protein; (9) method (M9) for allowing progress of a  
therapeutic regimen designed to alleviate a condition of abnormal  
expression of a gene product of (I); (10) method (M10) for  
determining regression, progression or onset of a pathological disorder  
characterized by a dysfunctional signal transduction; (11) pharmaceutical  
composition comprising the polypeptide of (2), a carrier, diluent or  
excipient; (12) method (M12) for preventing or delaying onset of a  
condition associated with reduced or non-existent levels of human (II) in  
a subject prone to the disorder; (13) method (M13) for monitoring the  
efficacy of an agent in correcting an abnormal level of human (II); (14)  
method (M14) for detecting a binding partner for human (II) in a sample  
suspected of containing the binding partner; (15) method (M15) of  
modulating the endogenous signal transducing activity of (II) in a  
mammal; (16) method (M16) for identifying a ligand that activates a  
receptor protein; (17) method (M17) for screening for a disorder  
characterized by expression of a dysfunctional human (II) coded  
for by a cDNA with a sequence substantially homologous to 1197 bp, given  
in the specification; (18) method (M18) for identifying agonist or  
antagonist of human (II); (19) host cell transfected with an isolated  
nucleic acid molecule comprising a sequence of nucleotides or  
ribonucleotides that encodes human (II); (20) recombinant non-human cell  
line which has been engineered to express a heterologous



protein; (21) isolated cell transformed or transfected with a sequence of nucleotides or ribonucleotides under conditions favoring cell surface expression of a functional human or mouse (II); (22) expression vector; (23) isolated nucleic acid molecule comprising a sequence of nucleotides that encodes a mouse (II); (24) isolated nucleic acid molecule comprising a coding region that encodes a splice variant of a mouse (II), where it is encoded by a fully defined sequence of 1191 bp, given in the specification; (25) isolated nucleic acid molecule that encodes a mouse (II) having a fully defined sequence of 396 amino acids, given in the specification; (26) isolated polypeptide encoded by the a sequence that is a splice variant of an isolated nucleic acid molecule that encodes a fully defined sequence of 396 amino acids, given in the specification; (27) isolated cells comprising the nucleic acid molecule of (23); (28) isolated mouse (II) encoded by (I); and (29) antibody that is specific for (II). 4787

WIDER DISCLOSURE - Also disclosed as new are kits comprising the compositions of the present invention, reagents, probes, primers, labels and a container.

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) and the nucleic acid molecule of (24) is preferably a genomic DNA, mRNA or cDNA. (I) further comprises a nucleotide sequence encoding a polypeptide which has at least 80% identity to a sequence of 398 amino acids, fully defined in the specification, which may include up to Na amino acid alterations over the entire length of the same sequence, where Na is the maximum number of amino acid alterations, and is calculated by Formula A,  $(A) Xa = 398$  amino acids, and  $Y = 0.80$ , where any non-integer product of Xa and Y is rounded down to the nearest integer prior to subtracting the product from Xa. The nucleic acid molecule of (23) that encodes a mouse (II) further comprises: (a) fully defined sequence of 1191 bp, given in the specification; (b) sequence that hybridizes under conditions of high stringency to the complement of the sequence in (a), and if it is DNA, a full complement or an RNA, is identical to mRNA native to a human cell; or (c) degenerate sequence of (a) or (b). Preferred Methods: (M5) comprises introducing (I) into a suitable host cell that expresses a functional (II), and assaying for second messenger activity in the cells. (M6) comprises introducing (I) into eukaryotic cells, and detecting second messenger activity in the cells, where the activity is mediated by a polypeptide encoded by (I). The sample in the (M8) is a biological tissue. The probe in (M10) is an antibody, where it is labeled with a radioactive label or enzyme. (M7) comprises contacting all or part of a fully defined sequence of 1197 bp, given in the specification, with the sample under conditions allowing a complex to form between the nucleic acid sequence and the mRNA, detecting the complexes, and determining the level of mRNA. The (M8) comprises: (a) measuring the second messenger activity of eukaryotic cells transformed with DNA encoding human (II) in the absence or presence of the test compound, thereby obtaining a first and second measurement, respectively; and (b) comparing the first and second measurements and identifying those compounds that result in a difference between the two measurements as a test compound that modulates the activity of human (II), where the eukaryotic cells express a functional human parathyroid hormone-2 receptor protein. The (M9) comprises: (a) assaying a sample from a subject to determine the level of a polypeptide encoded by a fully defined sequence of 1197 bp, or a polypeptide with a fully defined sequence of 398 amino acids, both given in the specification, at a first time point; (b) assaying level of the parameter in (a) at a second time point; and (c) comparing the level at the second time point to the level determined in (a) as a determination of effect of the therapeutic regimen. (M10) comprises incubating a sample with a complimentary nucleic acid hybridization probe with a sequence substantially homologous to 1197 bp, given in the specification, or with a detectable probe that is specific for the gene product of (I) under conditions favoring formation of a probe/gene product complex, and determining binding between the probe and any complimentary mRNA or a

formation of a probe/gene product complex that may be present as determinative of the regression, progression or onset of the pathological disorder. (M12) comprises administering (II) to the subject to prevent or delay onset of the condition. (M13) comprises administering the agent and determining a level of (II) following its administration, where a change in the level towards a normal level is indicative of the efficacy of the agent. (M14) comprises contacting with human (II) under conditions favoring binding of the receptor to the binding partner, and determining the presence of the binding partner in the sample by detecting binding of the receptor to the binding partner. (M15) comprises administering the binding partner detected in M(14). (M16) comprises: (a) introducing (II) into receptor protein-deficient cells or contacting endogenous-receptor protein-deficient host cells with candidate ligand, where the cells contain a reporter gene functionally linked to a hormone response element responsive to and induces expression of the reporter gene; (b) challenging the cells with candidate ligand which can potentially bind with the ligand-binding domain of the receptor protein; and (c) monitoring induction of the reporter gene, thereby identifying ligand that activate the receptor protein. (M17) comprises contacting a sample from a subject believed to suffer from the disorder with an antibody specific for an expression product of the same sequence, or contacting a cDNA or mRNA containing sample with a nucleic acid hybridization probe which hybridizes to a cDNA molecule comprising a fully defined sequence of 1197 bp, given in the specification, and determining binding between the antibody and the expression production as an indication of possible presence of the disorder in the subject. (M18) comprises contacting a cell expression on the surface the receptor protein, where the protein is associated with a second component capable of providing a detectable signal in response to the binding of a compound to the receptor, with a compound to be screened under conditions favoring binding of the compound to the receptor protein, and determining whether the compound binds to and activates or inhibits the receptor protein by measuring the level of a signal generated from the interaction of the compound with the receptor protein. Preferred Cell Line: The cell line further comprises a host cell transformed or transfected with a heterologous nucleic acid molecule comprising a sequence of nucleotides or ribonucleotides that encodes human (II). Preferred Isolated Cells: The isolated cells of (3) and (27) are bacterial cells, mammalian cells or amphibian oocytes, and the molecule is heterologous to the cells. Preferred Vector: The vector of (24) comprises (I) operably linked to a regulatory nucleotide sequence the controls expression of the nucleic acid molecule in a host cell. Preferred Antibody: The antibody of (29) is a monoclonal antibody. Preparation: (I) was prepared by standard recombinant techniques.

ACTIVITY - Antidiabetic; Analgesic, Vasotropic; Antimigraine; Antidepressant; Nootropic; Neuroprotective; Tranquilizer. Test details are described but no results are given.

MECHANISM OF ACTION - Gene Therapy; G-Protein-Agonist; G-Protein-Antagonist. No supporting data provided.

USE - The polypeptide is useful for preventing and/or treating abnormal conditions associated to both an excess of and insufficient amounts of human GPR54 activity, such as eating disorders, diabetes, pain, migraine, anxiety, depression, ischemia, Alzheimer's disease and reproductive and sleep disorders. also The polypeptide can be used to identify compounds which modulate its activity, and to follow progress of a therapeutic regime. The polynucleotide can be used to screen for a disorder or to determine regression, progression or onset of such a disorder, characterized by expression of a dysfunctional human GPR54 receptor protein (all claimed).

ADMINISTRATION - Dosage range of the compositions are from 0.1-100 mug/kg body weight, and the routes of administration include intravenous, subcutaneous, intramuscular, intraperitoneal, transmucosal, transdermal, oral and topical.

EXAMPLE - A piece of human genomic DNA encoding human G

protein-coupled receptor (GPR54) protein was utilized to generate a fragment for plasmid subcloning using specific oligonucleotide primers. First-round PCR was carried out using the primers pair HGPR54.F5 and HGPR54.R7 with the DNA polymerase Taq Gold and the DNA template Marathon-ready human hypothalamus cDNA in the presence of 5% DMSO for 40 cycles. The resulting PCR product was then used as a template for a second-round PCR under the same conditions except only 35 cycles were performed. The resulting (approximately 1300 bp) PCR product from this nested PCR was purified and cloned in to the vector pCR3.1. Clones containing full-length GPR54 were thus identified. (125 pages)

L17 ANSWER 11 OF 44 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 2002-17835 BIOTECHDS

TITLE: Isolated polynucleotide encoding human TIP39 peptide for identifying functional TIP39 and a test compound which modulates the activity of the peptide;  
vector-mediated recombinant protein gene transfer and expression in host cell for use in drug screening and gene therapy

AUTHOR: WANG H; KOBLAN K S; SUN H; DELLA PENNA K

PATENT ASSIGNEE: MERCK and CO INC

PATENT INFO: WO 2002033049 25 Apr 2002

APPLICATION INFO: WO 2000-US31954 17 Oct 2000

PRIORITY INFO: US 2000-241012 17 Oct 2000

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-471397 [50]

AB DERWENT ABSTRACT:

NOVELTY - An isolated polynucleotide (I) comprising a fully defined 572 base pair sequence (S1) encoding a human tuberoinfundibular peptide with a fully defined 39 amino acid sequence, both given in the specification or a sequence that hybridizes to the complement of S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid molecule (II) comprising a coding region of a splice variant of human tuberoinfundibular peptide (TIP39) encoded by S1; (2) isolated mammalian, bacterial or amphibian oocyte cells (III) comprising (I); (3) an isolated human TIP39 (IV) encoded by (I); (4) a cell membrane preparation (V) comprising (IV) with a sequence (S2) of SLALADDAAFRERARLLAALERRHWLNSYMHKLLVLDAP produced by a cell that expresses a recombinant expression vector encoding (IV); (5) a recombinant expression sequence (VI) encoding (IV) and comprising S1 which expresses the peptide in a transformed culture of eukaryotic or prokaryotic cells; (6) a cell culture (VII) transformed with (VI); (7) an expression vector (VIII) comprising (II) operably linked to a regulatory nucleotide sequence that controls expression of (II) in a host cell; (8) following (M1) the progress of a therapeutic regime designed to alleviate a condition characterized by abnormal expression of a gene product of (I) involving assaying a sample to determine the level of a parameter selected from a polypeptide encoded by S1 and S2 between 2 time points to determine the effect of the therapeutic regime; (9) determining (M2) regression, progression or onset of a pathological disorder characterized by a dysfunctional signal transduction, involving incubating a sample with: (a) a complementary probe homologous to a fully defined 100 amino acid sequence given in the specification and determining binding between the probe and any complementary messenger ribonucleic acid (mRNA) that may be present in the sample or (b) a detectable probe that is specific for the gene product of (I); (10) a pharmaceutical composition (IX) comprising (IV); (11) monitoring (M3) the efficacy of an agent in correcting abnormal levels of (IV); (12) screening (M4) and identifying agonists of (IV), by contacting a cell line that expresses the human parathyroid hormone-2 (PTH2) receptor with a test compound in the presence and in the absence of (IV) and determining whether, in the presence of (IV), the test compound inhibits



the binding of (IV) to a cell surface receptor in the cell line and if the test compound mimics the cellular effects of (IV) on the cell line, in which agonists are identified as those test compounds that inhibit the binding but mimic the cellular effects of (IV) on the cell line; (13) screening (M5) and identifying antagonists of (IV), by contacting a cell line that expresses the human PTH2 receptor with a test compound in the presence of (IV), and determining whether the test compound inhibits the binding and cellular effects of (IV) on the cell line, in which antagonists are identified as those compounds that inhibit both the binding and cellular effects of (IV) on the cell line; (14) screening (M6) for a disorder characterized by expression of a dysfunctional TIP39, coded for by a cDNA comprising a sequence of nucleotides substantially homologous to S1, involves: (a) contacting a sample from a subject believed to suffer from the disorder with an antibody specific for an expression product of S1, and determining binding between the antibody and the expression production as an indication of possible presence of the disorder in the subject; or (b) contacting a cDNA or mRNA containing sample from a subject with a nucleic acid hybridization probe which hybridizes to a cDNA molecule comprising S1, and determining binding of the hybridization probe to the cDNA or mRNA as an indication of possible presence of the disorder in the subject; (15) inhibiting (M7) binding of TIP39 to a cell presenting PTH2 receptor by adding an amount of an antagonist identified by M5 to a sample containing the cell in an amount sufficient to inhibit binding of the TIP39 to the cell; and (16) an antibody (X) that is specific for (IV).

WIDER DISCLOSURE - Disclosed are: (A) a plasmid containing (I); (B) a nucleic acid probe comprising nucleic acid molecules of sufficient length which specifically hybridize (I); (C) a diagnostic assay for detecting diseases related to mutations in (I); (D) a therapeutic composition comprising an immunological active or biological active fragments of (IV) or an antibody having affinity for (IV); (E) identifying cells that express (IV); (F) a nucleic acid having substantially the same sequence of S1; (G) a nucleic acid which differ from S1, but which has the same phenotype; (H) an antisense oligonucleotide having a sequence capable of binding specifically with any portion of mRNA that encodes (IV); (I) a transgenic non-human mammal capable of expressing (I); (J) diagnosing disease states characterized by abnormal signal transduction; and (K) a diagnostic system preferably in kit form comprising (I) in suitable packaging material.

BIOTECHNOLOGY - Preparation: (IV) is prepared by standard recombinant techniques. Preferred Nucleic Acid: (I) is genomic DNA, mRNA or cDNA. Preferred Method: In M2, the sample is a tissue. The probe is an antibody, where the antibody is labeled with a radioactive label or an enzyme. Preferred Antibody: (X) is a monoclonal antibody.

ACTIVITY - None given.

MECHANISM OF ACTION - Modulator of the endogenous signal transducing activity of a parathyroid hormone-2 (PTH2) receptor. No supporting data is given.

USE - (I) is useful for identifying functional TIP39, by introducing (I) into a suitable host cell that expresses a functional PTH2 receptor, and assaying for PTH2 receptor activity in the cells, or by introducing (I) into eukaryotic cells, and detecting PTH2 receptor activity in the cells, where the activity is mediated by a polypeptide encoded by the introduced nucleic acid molecule. (I) is useful for identifying DNA sequences encoding a TIP39, by probing a cDNA library or a genomic library with a labeled probe, preferably (I) comprising S1, and recovering from the library those sequences having a significant degree of homology relative to the probe. (I) is useful for detecting TIP39 messenger RNA in a biological sample by contacting all or part of S1 with the biological sample under conditions allowing a complex to form between the nucleic acid sequence and the messenger RNA, detecting the complexes, and determining the level of the messenger RNA. (I) is useful in a



bioassay for identifying a test compound, which modulates the activity of TIP39. (IV) is useful for preventing or delaying onset of a condition associated with reduced or non-existent levels of (IV), for detecting a binding partner for (IV) in a sample suspected of containing the binding partner, and for modulating the endogenous signal transducing activity of a PTH2 receptor in a mammal (claimed). (I) or (IV) is useful for in vitro purposes such as synthesis of DNA and manufacture of DNA vectors. (I), (IV) or (X) is useful as diagnostics, for distinguishing disease states caused by a dysfunctional endogenous TIP39 or PTH2 receptor, and for screening compounds in vitro to determine whether a compound functions as a potential agonist or antagonist to (IV). (X) is useful in immunohistochemistry techniques, for diagnostic and therapeutic applications, for purifying or detecting (IV), and for modulating the activity of (IV) in living animals or humans (all claimed).

EXAMPLE - A complementary deoxyribonucleic acid (cDNA) fragment specific to human tuberoinfundibular peptide (TIP39) was generated by polymerase chain reaction (PCR) amplification of human hypothalamus cDNA. The degenerate oligonucleotide primers (i) and (ii) were utilized to generate a fragment for plasmid subcloning. The resulting 98 base pair fragment was subcloned into the PCR II vector. Sequence analysis indicated that the fragment encoded a peptide that aligned with bovine TIP39 peptide between positions 5-36. The sequence information obtained from this clone was utilized to design the following oligonucleotide primer pairs, which yielded a PCR fragment of approximately 70 bp. Hw60 and KB01 primers were used to screen a human fetal brain stem cDNA library. Two identical clones were identified and sequenced. Hw37 TIGCIGA(T/C)GA(T/C)GCIGCITTCCG (i); and Hw39 TCIA(A/G)IACIA(A/G)IA(A/G)IA(A/G)(C/T)TTGTGCAT (ii). (61 pages)

L17 ANSWER 12 OF 44 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2002268325 EMBASE  
 TITLE: Transcript expression of the tuberoinfundibular peptide (TIP)39/PTH2 receptor system and non-PTH1 receptor-mediated tonic effects of TIP39 and other PTH2 receptor ligands in renal vessels.  
 AUTHOR: Eichinger A.; Fiaschi-Taesch N.; Massfelder T.; Fritsch S.; Barthelmebs M.; Helwig J.-J.  
 CORPORATE SOURCE: Dr. J.-J. Helwig, Pharmacol./Physiol. Renovasculaires, E. M. I. N. S. Rec. M.-U. L. Pasteur, Batiment 4, 11 rue Humann, F67085 Strasbourg Cedex, France.  
 SOURCE: jean-jacques.helwig@pharmaco-ulp.u-strasbg.fr  
 Endocrinology, (2002) Vol. 143, No. 8, pp. 3036-3043. .  
 Refs: 43  
 ISSN: 0013-7227 CODEN: ENDOAO  
 COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article  
 FILE SEGMENT: 002 Physiology  
 003 Endocrinology  
 LANGUAGE: English  
 SUMMARY LANGUAGE: English  
 ENTRY DATE: Entered STN: 8 Aug 2002  
 Last Updated on STN: 8 Aug 2002

AB Although lower than in brain, the type 2 PTH receptor (PTH2-R) has been shown to be expressed throughout the cardiovascular system. Tuberoinfundibular peptide (TIP) purified from brain is thought to be the endogenous selective ligand of the PTH2-R. In the present studies, TIP and PTH2-R mRNA expressions were evidenced by RT-PCR in rat intrarenal arteries as well as in renovascular smooth muscle cells cultured from these arteries. In the isolated perfused rat kidney (IPK), peptides known to bind to both PTH1- and PTH2-Rs, such as rat PTH (1-34) and the hybrid PTH/PTHrP peptide, [Ile(5), Trp(23)]PTHrP (1-36), failed to exhibit improved vasodilatory effect, compared with human PTHrP (1-36), which binds only to the PTH1-R. Thus, a non-PTH1-R seemed not to be

involved in the vasodilatory effects of these peptides. On the other hand, TIP exhibited complex vasoactivity, constricting the IPK at 10 nM and dilating the IPK at 1, 100, and 1000 nM. Moreover, [p-benzoyl-L-Phe(4),Ile(5),Trp(23)]PTHrP (1-36), initially described as a selective PTH2-R antagonist, also displayed a strong vasodilatory effect and therefore could not be used to check that TIP-induced vasoactivity was mediated by the PTH2-R. However, both [p-benzoyl-L-Phe(4),Ile(5),Trp(23)]PTHrP (1-36) and TIP displayed similar or even enhanced vasodilation in IPK in which PTH1-R-induced vasodilation was fully desensitized by sustained exposure to human PTHrP (1-36). Importantly, in IPK desensitized to the vasodilatory action of PTHrP (1-36), the hybrid PTH/PTHrP peptide and rat PTH (1-34), whose vasodilatory responses appeared exclusively PTH1-R dependent in naive IPK, produced a new and strong vasodilation. In conclusion, TIP and PTH2-R mRNAs are expressed in renal vessels and TIP appears as a new vasoactive peptide. Whether TIP interacts with PTH2-R could not be shown. However, these studies reveal the ability of TIP, as well as of other peptides known to bind to the PTH2-R, to dilate renal vessels in a PTH1-R-independent manner. Moreover, results obtained in IPK desensitized to the vasodilatory action of PTHrP (1-36) strongly suggest that TIP, along with PTHrP, might be coordinately involved in the regulation of renal hemodynamics.

L17 ANSWER 13 OF 44 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2002:124659 HCAPLUS

DOCUMENT NUMBER: 136:289223

TITLE: Anatomical and physiological evidence for involvement of tuberoinfundibular peptide of 39 residues in nociception

AUTHOR(S): Dobolyi, Arpad; Ueda, Hiroshi; Uchida, Hitoshi; Palkovits, Miklos; Usdin, Ted B.

CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, Bethesda, MD, 20892, USA

SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2002), 99(3), 1651-1656  
CODEN: PNASA6; ISSN: 0027-8424

PUBLISHER: National Academy of Sciences

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The parathyroid hormone 2 (PTH2) receptor's anatomical distribution suggests that, among other functions, it may be involved in modulation of nociception. We localized PTH2 receptor protein to spinal cord lamina II and showed that it is synthesized by subpopulations of primary sensory neurons and intrinsic spinal cord dorsal horn neurons. Tuberoinfundibular peptide of 39 residues (TIP39) selectively activates the PTH2 receptor. Intraplantar micro-injection of TIP39 caused a paw-withdrawal response and intrathecal injection caused scratching, biting, and licking, a nocifensive response. Intrathecal administration of a TIP39 antibody decreased sensitivity in tail-flick and paw-pressure assays. Intrathecal administration of TIP39 potentiated responses in these assays. We determined the sequence of TIP39's precursor and found that mRNA encoding TIP39 and TIP39-like immunoreactivity is concentrated in two brainstem areas, the subparafascicular area and the caudal paralemniscal nucleus. Cells in these areas project to the superficial dorsal horn of the spinal cord. Our data suggest that TIP39 released from supraspinal fibers potentiates aspects of nociception within the spinal cord.

REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L17 ANSWER 14 OF 44 LIFESCI COPYRIGHT 2006 CSA on STN

ACCESSION NUMBER: 2003:77906 LIFESCI

TITLE: Expression and Distribution of Tuberoinfundibular Peptide of 39 Residues in the Rat Central Nervous System

AUTHOR: Dobolyi, A.; Palkovits, M.; Usdin, T.B.  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, 36 Convent Dr., Bethesda, MD 20892-4094, USA; E-mail: usdin@codon.nih.gov  
SOURCE: Journal of Comparative Neurology [J. Comp. Neurol.], (2002) vol. 455, no. 4, pp. 547-566. ISSN: 0021-9967.  
DOCUMENT TYPE: Journal  
FILE SEGMENT: N3  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB Tuberoinfundibular peptide of 39 residues (TIP39) has been recently purified and identified as a selective ligand for the parathyroid hormone 2 receptor. As a next step toward understanding its functions, we report the expression and distribution of TIP39 in the rat central nervous system. In situ hybridization histochemistry and immunocytochemistry revealed TIP39-containing cell bodies in three distinct areas. The major one comprises the subparafascicular area posterior through the intralaminar nucleus of the thalamus; a second is the medial paralemniscal nucleus at the pontomesencephalic junction; and a third is in the dorsal and dorsolateral hypothalamic areas, which contained a few, scattered cell bodies. We found, in contrast to the highly restricted localization of TIP39-containing cell bodies, a much more widespread localization of TIP39-containing fibers. The highest density of fibers was observed in limbic areas such as the septum, the amygdala, and the bed nucleus of the stria terminalis; in areas involved in endocrine regulation, such as the hypothalamic dorsomedial, paraventricular, periventricular, and arcuate nuclei; in auditory areas, such as the ectothalamic and temporal cortices, inferior colliculus, medial geniculate body, and some of the nuclei of the superior olivary complex; and in the dorsolateral funiculus of the spinal cord. The localization of TIP39-containing nuclei and fibers provides an anatomical basis for previously demonstrated endocrine and nociceptive effects of TIP39 and suggests additional functions for TIP39, one apparent candidate being the regulation of auditory information processing.

L17 ANSWER 15 OF 44 MEDLINE on STN DUPLICATE 5  
ACCESSION NUMBER: 2003023951 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 12529938  
TITLE: The parathyroid hormone 2 (PTH2) receptor.  
AUTHOR: Usdin T B; Bonner T I; Hoare S R J  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, Building 36/Room 3D06, 36 Convent Drive, Bethesda, Maryland 20892-4094, USA.. usdin@codon.nih.gov  
SOURCE: Receptors & channels, (2002) Vol. 8, No. 3-4, pp. 211-8. Ref: 36  
Journal code: 9315376. ISSN: 1060-6823.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200305  
ENTRY DATE: Entered STN: 18 Jan 2003  
Last Updated on STN: 15 May 2003  
Entered Medline: 14 May 2003  
AB The human PTH2 receptor binds and is activated at high potency by PTH and by the recently discovered peptide tuberoinfundibular peptide of 39 residues (TIP39). Rat and zebrafish PTH2 receptors are more weakly activated by PTH, suggesting that TIP39 may be the natural ligand for the PTH2 receptor. Unlike the PTH1 receptor, the PTH2 receptor interacts extremely weakly with parathyroid hormone-related peptide (PTHrP). The PTH2 receptor is strongly coupled to stimulation of cAMP accumulation, and

more weakly, in a cell-specific manner to increases in intracellular calcium concentration. A variety of evidence supports the general model of receptor amino terminal sequences binding ligand carboxyl terminal sequences with high affinity, and ligand amino terminal sequences activating the receptor through interaction with the "juxtamembrane" portion of the receptor. The receptor is present at greatest levels in the nervous system. It is expressed in scattered cells in the cerebral cortex and basal ganglia and at relatively high abundance in the septum, midline thalamic nuclei, several hypothalamic nuclei, and the dorsal horn of the spinal cord. Peripherally, expression in pancreatic islet somatostatin cells is most dramatic. Functional hypotheses based on the receptor's distribution are being tested. Recent data support involvement in hypothalamic releasing-factor secretion and pain.

L17 ANSWER 16 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:468730 BIOSIS  
DOCUMENT NUMBER: PREV200200468730  
TITLE: Characterization of the human and mouse genes encoding the tuberoinfundibular peptide of 39 residues, a ligand of the parathyroid hormone receptor family.  
AUTHOR(S): Hansen, I. A.; Jakob, O.; Wortmann, S.; Arzberger, T.; Allolio, B.; Blind, E. [Reprint author]  
CORPORATE SOURCE: Department of Medicine (Endocrinology), University of Wuerzburg, Josef-Schneider-Strasse 2, D-97080, Wuerzburg, Germany  
SOURCE: eberhard.blind@mail.uni-wuerzburg.de  
Journal of Endocrinology, (July, 2002) Vol. 174, No. 1, pp. 95-102. print.  
CODEN: JOENAK. ISSN: 0022-0795.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
OTHER SOURCE: Genbank-AC083740; EMBL-AC083740; DDBJ-AC083740; Genbank-AY037555; EMBL-AY037555; DDBJ-AY037555  
ENTRY DATE: Entered STN: 4 Sep 2002  
Last Updated on STN: 31 Oct 2002

AB The polypeptide TIP39 (tuberoinfundibular peptide of 39 residues) is a potent activator of the parathyroid hormone (PTH)-2 receptor (P2R) and an antagonist of the PTH-1 receptor (P1R). To clarify its possible physiological function(s), we studied its interaction with the human P1R and P2R and examined the expression of TIP39 in man and mouse. To find out possible sites of this ligand interaction in the organism, we identified the genes encoding the TIP39 protein precursors of Homo sapiens and Mus musculus in the databases of the human and mouse genome projects respectively. We then obtained the full-length cDNAs of both species by RACE-PCR. The deduced TIP39 preprohormones consist of an N-terminal 30 amino acid (aa) signal peptide followed by a 29 aa TIP39 precursor-related peptide, an Arg-Arg processing site, and the actual 39 aa TIP39 sequence. The first 23 aa of the actual TIP39 sequence, thought to contain the P2R receptor activation site, are identical in man and mouse and thus phylogenetically conserved. By contrast, the 16 aa C-terminal portion showed a higher degree of diversity (75% aa identity). By using RT-PCR, TIP39 was found to be highly expressed in human central nervous system tissues, trachea, fetal liver, and, to a lesser degree, in human heart and kidney. Using in situ hybridization, TIP39 mRNA expression was revealed in various areas of the mouse brain. In a homologous human cell model using human embryonic kidney 293 cells stably transfected with human P1R and P2R, human TIP39 did bind to P1R with moderate affinity ( $IC_{50}$  approx  $10^{-7}$ - $10^{-6}$  M), but showed higher affinity binding to P2R ( $IC_{50}$  approx  $10^{-8}$  M), comparable to the affinity of human N-terminal PTH (hPTH(1-34)) to this receptor. In P2R-transfected cells, the cAMP pathway was activated more efficiently (approx 10-fold) by TIP39 as a ligand compared to hPTH(1-34). In P1R-transfected cells, only



hPTH(1-34) but not TIP39 was able to elicit a cAMP response, but TIP39 was able to directly antagonize the cAMP-stimulating effect of hPTH(1-34) on this receptor. In conclusion, we could show a possible function of TIP39 for the human organism as a potent activator of P2R (e.g. in brain) as well as an antagonist of the action of PTH and/or PTH-related protein on P1R (e.g. in bone and kidney). The physiological role of TIP39 in calcium metabolism with regard to these actions remains to be determined. The tools developed in this work will allow us to investigate the possible role of TIP39 as a locally or systemically secreted ligand modulating the function of the PTH receptor family.

L17 ANSWER 17 OF 44 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN

ACCESSION NUMBER: 2001211809 EMBASE  
TITLE: Extracts from tumors causing oncogenic osteomalacia inhibit phosphate uptake in opossum kidney cells.  
AUTHOR: Jonsson K.B.; Mannstadt M.; Miyauchi A.; Yang I.M.; Stein G.; Ljunggren O.; Juppner H.  
CORPORATE SOURCE: K.B. Jonsson, Massachusetts General Hospital, Endocrine Unit, Harvard Medical School, 50 Blossom Street, Boston, MA 02114-2698, United States. Kenneth.Jonsson@medicin.uu.se  
SOURCE: Journal of Endocrinology, (2001) Vol. 169, No. 3, pp. 613-620. .  
Refs: 29  
ISSN: 0022-0795 CODEN: JOENAK  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 003 Endocrinology  
016 Cancer  
029 Clinical Biochemistry  
033 Orthopedic Surgery  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 10 Jul 2001  
Last Updated on STN: 10 Jul 2001

AB In oncogenic osteomalacia (OOM), a tumor produces an unknown substance that inhibits phosphate reabsorption in the proximal tubules. This causes urinary phosphate wasting and, as a consequence, hypophosphatemic osteomalacia. To characterize this poorly understood biological tumor activity we generated aqueous extracts from several OOM tumors. Extracts from three of four tumors inhibited, dose- and time-dependently, (32)P orthophosphate uptake by opossum kidney (OK) cells; maximum inhibition was about 45% untreated control. Further characterization revealed that the factor is resistant to heat and several proteases, and that it has a low molecular weight. The tumor extracts also stimulated cAMP accumulation in OK cells, but not in osteoblastic ROS 17/2.8 and UMR 106 cells, or in LLC-PK1 kidney cells expressing the parathyroid hormone (PTH)/PTH-related peptide receptor or the PTH-2 receptor. HPLC separation of low molecular weight fractions of the tumor extracts revealed that the flow-through of all three positive tumor extracts inhibited (32)P uptake and stimulated cAMP accumulation in OK cells. Additionally, a second peak with inhibitory activity on phosphate transport, but without cAMP stimulatory activity, was identified in the most potent tumor extract. We have concluded that several low molecular weight molecules with the ability to inhibit phosphate transport in OK cells can be found in extracts from OOM tumors. It remains uncertain, however, whether these are related to the long-sought phosphaturic factor responsible for the phosphate wasting seen in OOM patients.

L17 ANSWER 18 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2001:571296 BIOSIS  
DOCUMENT NUMBER: PREV200100571296  
TITLE: Studies on expression and regulation of

parathyroid hormone 2-receptor  
on human leucocytes in hyperparathyroidism.  
AUTHOR(S): Kosch, M. [Reprint author]; Seeliger, S. [Reprint author];  
Usdin, T.; Rahn, K. H. [Reprint author]; Hausberg, M.  
[Reprint author]  
CORPORATE SOURCE: Department of Internal Medicine D, University of Muenster,  
Muenster, Germany  
SOURCE: Kidney and Blood Pressure Research, (2001) Vol. 24, No.  
4-6, pp. 307. print.  
Meeting Info.: Joint Scientific Meeting of the Nephrology  
Society and the German Working Group for Clinical  
Nephrology. Munster, Germany. September 29-October 02,  
2001.  
ISSN: 1420-4096.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 12 Dec 2001  
Last Updated on STN: 25 Feb 2002

L17 ANSWER 19 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2001:246727 BIOSIS  
DOCUMENT NUMBER: PREV200100246727  
TITLE: Parathyroid hormone 2  
-receptor is expressed on human leucocytes and  
down-regulated in hyperparathyroidism.  
AUTHOR(S): Kosch, M.; Hausberg, M.; Eue, I. [Reprint author]; Usdin,  
T.; Seeliger, S. [Reprint author]  
CORPORATE SOURCE: Institut fuer Physiologie, Robert-Koch-Str. 27a, D-48149,  
Muenster, Germany  
SOURCE: Pfluegers Archiv European Journal of Physiology, (2001)  
Vol. 441, No. 6 Supplement, pp. R156. print.  
Meeting Info.: Joint Congress of the Scandinavian and the  
German Physiological Societies. Berlin, Germany. March  
10-13, 2001.  
CODEN: PFLABK. ISSN: 0031-6768.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
Conference; (Meeting Poster)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 23 May 2001  
Last Updated on STN: 19 Feb 2002

L17 ANSWER 20 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on  
STN

ACCESSION NUMBER: 2001:486661 BIOSIS  
DOCUMENT NUMBER: PREV200100486661  
TITLE: Distribution of mRNA encoding the peptide neuromodulator  
candidate TIP39 in the nervous system.  
AUTHOR(S): Dobolyi, A. [Reprint author]; Palkovits, M. [Reprint  
author]; Mezey, E.; Usdin, T. B. [Reprint author]  
CORPORATE SOURCE: Lab. Genetics, NIMH, NIH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstracts, (2001) Vol. 27, No. 1,  
pp. 70. print.  
Meeting Info.: 31st Annual Meeting of the Society for  
Neuroscience. San Diego, California, USA. November 10-15,  
2001.  
ISSN: 0190-5295.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 17 Oct 2001  
Last Updated on STN: 23 Feb 2002

AB We recently purified tuberoinfundibular peptide of 39 residues (TIP39) from bovine hypothalamus and demonstrated that it is a potent and selective parathyroid hormone 2 (PTH2) receptor agonist. Our previous observation that the PTH2 receptor's CNS distribution is topographically widespread suggests that TIP39 may be involved in a variety of neural functions. As the next step toward defining its roles, we have begun to examine TIP39's distribution. We identified TIP39's human and mouse genomic sequences and used them to predict its mRNA sequence, which we confirmed in rat and mouse by RT-PCR. Using RT-PCR as a screen, we found TIP39 mRNA in rat diencephalon, brainstem, cerebellum and dorsal root ganglia, but not in cerebral cortex, hippocampus and spinal cord. To investigate the distribution of TIP39 mRNA at the cellular level we performed in situ hybridization histochemistry (ISHH) on sections from rat brain and spinal cord using two non-overlapping 35S-labelled RNA TIP39 probes. In all 7 animals tested, two groups of intensely labeled cells were present: one in the most medial part of the central gray at the hypothalamic-midbrain junction; the other in the lateral pons bordered by the ventral lateral lemniscal and Kolliker-Fuse nuclei. We confirmed these ISHH observations by RT-PCR of microdissected tissue pellets. Other areas had much less intense labeling and are currently being analyzed. The fine topographical distribution of TIP39 neurons will facilitate experimental studies to clarify its function.

L17 ANSWER 21 OF 44 MEDLINE on STN DUPLICATE 6  
ACCESSION NUMBER: 2001132182 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11123392  
TITLE: The clinical impact of metabolic bone disease in coeliac disease.  
AUTHOR: Fickling W E; McFarlane X A; Bhalla A K; Robertson D A  
CORPORATE SOURCE: Department of Gastroenterology, Royal United Hospital, Bath, Avon BA1 3NG, UK.  
SOURCE: Postgraduate medical journal, (2001 Jan) Vol. 77, No. 903, pp. 33-6.  
JOURNAL CODE: 0234135. ISSN: 0032-5473.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200103  
ENTRY DATE: Entered STN: 4 Apr 2001  
Last Updated on STN: 4 Apr 2001  
Entered Medline: 1 Mar 2001

AB Bone mineral density was measured by dual energy x ray absorptiometry (DEXA) at the lumbar spine and femoral neck in 15 adults who had metabolic bone disease in association with coeliac disease (mean age at diagnosis 53.5 years, range 37 to 66). Results were expressed as a T score (the number of standard deviations by which patient's bone density differed from the sex matched young adult mean). Three patients had no skeletal symptoms and normal routine calcium biochemistry but severely reduced axial bone mineral density on DEXA. Eleven patients had symptomatic skeletal fractures, including fractures of proximal femur (3), vertebrae (4), and radius (6). Three patients had osteomalacia confirmed on bone biopsy, two of whom had characteristic biochemistry. Secondary and tertiary hyperparathyroidism were seen. Seventy five further patients (60 female) with coeliac disease (mean age 52.0 years, median duration of gluten-free diet 3.4 years) and 75 paired healthy age and sex matched controls were questioned on past fracture history. Patients with coeliac disease underwent detailed studies of calcium biochemistry, dietary intake, and bone mineral density. Sixteen had a past history of fractures ( $\chi^2 = 10.7$ ,  $p = 0.0004$ , v controls), which were of typical osteoporotic type. Ten patients had fracture before diagnosis of coeliac disease and six after diagnosis. Patients who had a fracture were older (56.3 v 50.3 years,  $p < 0.02$ , Wilcoxon rank sum test) than those with no

fracture. There was no significant difference in bone mineral density (z score -0.31 v -0.77), serum calcium (2.30 v 2.26 mmol/l), 25-hydroxyvitamin D (19.7 v 23.7 nmol/l), parathyroid hormone (2.6 v 3.1 pmol/l), or dietary calcium intake (1021.0 v 1033.0 mg/day) in patients with fracture compared with those without fracture. Metabolic bone disease is common in coeliac disease and is associated with premature osteoporotic fractures.

L17 ANSWER 22 OF 44 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:688877 SCISEARCH

THE GENUINE ARTICLE: 351EL

TITLE: Molecular determinants of tuberoinfundibular peptide of 39 residues (TIP39) selectivity for the parathyroid hormone-2 (PTH2) receptor - N-terminal truncation of TIP39 reverses PTH2 receptor/PTH1 receptor binding selectivity

AUTHOR: Hoare S R J; Clark J A; Usdin T B (Reprint)

CORPORATE SOURCE: NIH, Rm 3D06, Bldg 36, 36 Convent Dr MSC4094, Bethesda, MD 20892 USA (Reprint); NIMH, Cell Biol Unit, Genet Lab, Bethesda, MD 20892 USA

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1 SEP 2000) Vol. 275, No. 35, pp. 27274-27283.

ISSN: 0021-9258.

PUBLISHER: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 41

ENTRY DATE: Entered STN: 2000

Last Updated on STN: 2000

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Tuberoinfundibular peptide of 39 residues (TIP39) and the parathyroid hormone-a (PTH2) receptor form part of an extended family of related signaling molecules that includes the PTH1 receptor, which responds to PTH and PTH-related protein. TIP39 does not appreciably activate the PTH1 receptor, but in this study it is shown to bind the receptor with moderate affinity (59 nM). In this study, we investigated the molecular determinants of both ligand and receptor for the PTH2 receptor selectivity of TIP39 and quantitatively evaluated the role of molecular elements in the binding of TIP39 to the PTH2 and PTH1 receptors. A chimeric receptor composed of the N-terminal extracellular domain of the PTH1 receptor and the remainder (juxtamembrane domain) of the PTH2 receptor (P2-NP1) was fully activated by TIP39 (E-max = 98% of the rPTH-(1-34), E-max, EC50 = 2.0 nM). This receptor chimera bound TIP39 with an equivalent affinity to the wild-type PTH2 receptor (2.3 and 2.0 nM, respectively). The reciprocal chimeric receptor (P1-NP2) was not activated by TIP39 and bound the ligand with an affinity equivalent to that of the PTH1 receptor. Thus, the juxtamembrane receptor domain specifies the signaling and binding selectivity of TIP39 for the PTH2 receptor over the PTH1 receptor. Removing six N-terminal residues of TIP39 eliminated activation of the PTH2 receptor and reduced binding affinity 70-fold. In contrast, this truncation increased affinity for the PTH1 receptor 10-fold, reversing the PTH2/PTH1 receptor binding selectivity and resulting in a high affinity interaction of TIP-(7-39) with the PTH1 receptor (6 nM). These findings can be explained by a strong interaction between the N-terminal region of TIP39 and the juxtamembrane domain of the PTH2 receptor, with the corresponding domain of the PTH1 receptor acting as a selectivity barrier against high affinity binding of TIP39. As a result, TIP-(7-39) is a highly potent, selective antagonist for the PTH1 receptor.

L17 ANSWER 23 OF 44 EMBASE COPYRIGHT (c) 2006 Elsevier B.V. All rights reserved on STN



ACCESSION NUMBER: 2001129864 EMBASE  
TITLE: Evaluating the ligand specificity of zebrafish parathyroid hormone (PTH) receptors: Comparison of PTH, PTH-related protein, and tuberoinfundibular peptide of 39 residues.  
AUTHOR: Hoare S.R.J.; Rubin D.A.; Juppner H.; Usdin T.B.  
CORPORATE SOURCE: Dr. T.B. Usdin, Unit on Cell Biology, Laboratory of Genetics, National Institute of Mental Health, 36 Convent Drive, Bethesda, MD 20892-4094, United States.  
usdin@codon.nih.gov  
SOURCE: Endocrinology, (2000) Vol. 141, No. 9, pp. 3080-3086. .  
Refs: 22  
ISSN: 0013-7227 CODEN: ENDOAO  
COUNTRY: United States  
DOCUMENT TYPE: Journal; Article  
FILE SEGMENT: 003 Endocrinology  
030 Pharmacology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
ENTRY DATE: Entered STN: 19 Apr 2001  
Last Updated on STN: 19 Apr 2001  
AB Homologs of mammalian PTH1 and PTH2 receptors, and a novel PTH3 receptor have been identified in zebrafish (zPTH1, zPTH2, and zPTH3), zPTH1 receptor ligand specificity is similar to that of mammalian PTH1 receptors. The zPTH2 receptor is selective for PTH over PTH-related protein (PTHrP); however, PTH produces only modest cAMP accumulation. A PTH2 receptor-selective peptide, tuberoinfundibular peptide of 39 residues (TIP39), has recently been purified from bovine hypothalamus. The effect of TIP39 has not previously been examined on zebrafish receptors. The zPTH3 receptor was initially described as PTHrP selective based on comparison with the effects of human PTH. We have now examined the ligand specificity of the zebrafish PTH-recognizing receptors expressed in COS-7 cells using a wide range of ligands. TIP39 is a potent agonist for stimulation of cAMP accumulation at two putative splice variants of the zPTH2 receptor (EC(50), 2.6 and 5.2 nM); in comparison, PTH is a partial agonist [maximal effect (E(max)) of PTH peptides ranges from 28-49% of the TIP39 E(max)]. As TIP39 is much more efficacious than any known PTH-like peptide, a homolog of TIP39 may be the zPTH2 receptor's endogenous ligand. At the zPTH3 receptor, rat PTH-(1-34) and rat PTH-(1-84) (EC(50), 0.22 and 0.45 nM) are more potent than PTHrP (EC(50), 1.5 nM), and rPTH-(1-34) binds with high affinity (3.2 nM). PTH has not been isolated from fish. PTHrP-like peptides, which have been identified in fish, may be the natural ligands for zPTH1 and zPTH3 receptors.

L17 ANSWER 24 OF 44 MEDLINE on STN DUPLICATE 7  
ACCESSION NUMBER: 2001077468 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 11098126  
TITLE: Distribution of parathyroid hormone-2 receptor-like immunoreactivity and messenger RNA in the rat nervous system.  
AUTHOR: Wang T; Palkovits M; Rusnak M; Mezey E; Usdin T B  
CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National Institute of Mental Health, 36 Convent Drive MSC4094, Bethesda, MD 20892-4094, USA.  
SOURCE: Neuroscience, (2000) Vol. 100, No. 3, pp. 629-49.  
Journal code: 7605074. ISSN: 0306-4522.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200101  
ENTRY DATE: Entered STN: 22 Mar 2001  
Last Updated on STN: 22 Mar 2001  
Entered Medline: 11 Jan 2001  
AB The parathyroid hormone-2 receptor is a

member of the secretin family of guanine nucleotide-binding protein-coupled receptors. The human parathyroid hormone-2 receptor is activated by parathyroid hormone and a recently purified hypothalamic polypeptide, tubero-infundibular peptide of 39 residues, while the rat parathyroid hormone-2 receptor is poorly activated by parathyroid hormone and is potently activated by tubero-infundibular peptide of 39 residues. In order to provide a foundation for studies on the physiological role of the parathyroid hormone-2 receptor and tubero-infundibular peptide of 39 residues, we investigated the cellular distribution of the parathyroid hormone-2 receptor in the rat CNS using both immunohistochemistry and in situ hybridization histochemistry. The receptor is found in discrete groups of neurons in many regions. It is present in scattered small cells throughout the cerebral cortex, in small and medium-sized cells in the striatum, and is quite abundant in the septum and the midline thalamic nuclei. Its expression is high in the hypothalamus, particularly in the periventricular and arcuate nuclei. Fibers and terminals in the external zone of the median eminence, and in the superficial layers of the caudal spinal trigeminal tract and the spinal cord dorsal horn, are strongly and dramatically labeled by a parathyroid hormone-2 receptor-selective antibody. The localization of parathyroid hormone-2 receptor suggests a role in the regulation of pituitary hormone secretion, sensory information processing and homeostatic regulation.

L17 ANSWER 25 OF 44 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 2001034296 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11013069

TITLE: New members of the parathyroid hormone/parathyroid hormone receptor family: the parathyroid hormone 2 receptor and tuberoinfundibular peptide of 39 residues.

AUTHOR: Usdin T B; Wang T; Hoare S R; Mezey E; Palkovits M

CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland 20892-4094, USA..  
usdin@codon.nih.gov

SOURCE: Frontiers in neuroendocrinology, (2000 Oct) Vol. 21, No. 4, pp. 349-83. Ref: 160  
Journal code: 7513292. ISSN: 0091-3022.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
General Review; (REVIEW)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200011

ENTRY DATE: Entered STN: 22 Mar 2001  
Last Updated on STN: 22 Mar 2001  
Entered Medline: 30 Nov 2000

AB The parathyroid hormone (PTH) family currently includes three peptides and three receptors. PTH regulates calcium homeostasis through bone and kidney PTH1 receptors. PTH-related peptide, probably also through PTH1 receptors, regulates skeletal, pancreatic, epidermal, and mammary gland differentiation and bladder and vascular smooth muscle relaxation and has a CNS role that is under investigation. Tuberoinfundibular peptide of 39 residues (TIP39) was recently purified from bovine hypothalamus based on selective PTH2 receptor activation. PTH2 receptor expression is greatest in the CNS, where it is concentrated in limbic, hypothalamic, and sensory areas, especially hypothalamic periventricular neurons, nerve terminals in the median eminence, superficial layers of the spinal cord dorsal horn, and the caudal part of the sensory trigeminal nucleus. It is also present in a number of endocrine cells. Thus TIP39 and PTH2 receptor-influenced functions may range from pituitary and pancreatic hormone release to pain perception. A third PTH-recognizing receptor has

been found in zebrafish.  
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STN

ACCESSION NUMBER: 2001:75858 BIOSIS  
DOCUMENT NUMBER: PREV200100075858  
TITLE: Evidence for PTH2 receptor involvement in nociception.  
AUTHOR(S): Usdin, T. B. [Reprint author]; Palkovits, M.; Mezey, E.;  
Rusnik, M.  
CORPORATE SOURCE: NIMH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No.  
1-2, pp. Abstract No.-15.7. print.  
Meeting Info.: 30th Annual Meeting of the Society of  
Neuroscience. New Orleans, LA, USA. November 04-09, 2000.  
Society for Neuroscience.  
ISSN: 0190-5295.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 7 Feb 2001  
Last Updated on STN: 12 Feb 2002

AB The rat parathyroid hormone 2 receptor  
(PTH2-R) is activated by a recently discovered peptide, TIP39, that may be  
its endogenous ligand (Usdin et al, Nature Neurosci. 2, 941 1999). We  
previously localized the PTH2-R in several areas involved in nociception,  
including nerve terminals in the spinal cord superficial dorsal horn and  
caudal trigeminal nucleus, and cell bodies in the lateral parabrachial  
nucleus, midline thalamic nuclei and primary sensory ganglia. It is  
expressed in other areas, including relatively high levels in the  
hypothalamic periventricular nucleus (PeriVN). We are examining  
modulation of PTH2-R expression to evaluate its involvement in  
functional circuits. Rats were sacrificed one hour after injection of 4%  
formalin into a leg. In situ hybridization showed c-Fos mRNA induction  
with an established "pain pattern". One set of sections was hybridized  
with a PTH2-R probe. On visual inspection of emulsion autoradiograms  
PTH2-R mRNA expression was decreased in several brain areas,  
including the hypothalamic PeriVN and some midline thalamic nuclei, in  
animals receiving the painful stimulus. In the PeriVN of treated animals  
(N=6 for control and treatment) the area occupied by silver grains was 48%  
of control (p=.03), total optical density was 71% control (p=.02), the  
number of cells with a grain level over background was 75% of control  
(p=.028) and the area of labeling per cell was 66% control (p=.04). Thus,  
this painful stimulus decreases the amount of PTH2-R expression  
in PeriVN neurons and the number of neurons expressing it at  
detectable levels. Quantitation in other areas is underway. These data  
suggest a role for the PTH2-R and TIP39 in nociception.

L17 ANSWER 27 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on  
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ACCESSION NUMBER: 2001:135269 BIOSIS  
DOCUMENT NUMBER: PREV200100135269  
TITLE: Brain administration of tuberoinfundibular peptide of 39  
residues inhibits growth hormone secretion.  
AUTHOR(S): Wang, T. [Reprint author]; Edwards, G. L.; Lange, G. D.;  
Parlow, A. F.; Usdin, T. B.  
CORPORATE SOURCE: NIMH, Bethesda, MD, USA  
SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No.  
1-2, pp. Abstract No.-780.10. print.  
Meeting Info.: 30th Annual Meeting of the Society of  
Neuroscience. New Orleans, LA, USA. November 04-09, 2000.  
Society for Neuroscience.  
ISSN: 0190-5295.  
DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 14 Mar 2001

Last Updated on STN: 15 Feb 2002

AB We recently purified TIP39, a previously undescribed peptide from bovine hypothalamus. It is a potent and selective activator of both the rat and human parathyroid hormone 2 (PTH2) receptors and may be the PTH2 receptor's normal endogenous ligand. In the rat CNS the PTH2 receptor is highly expressed in the external layer of the median eminence, arcuate nucleus, and somatostatin-containing neurons in the hypothalamic periventricular nucleus. This localization suggests a role for TIP39 and the PTH2 receptor in regulation of anterior pituitary hormone secretion. We have now examined the hypothesis that TIP39 is involved in the regulation of growth hormone (GH) secretion by investigating the effect of intracerebroventricular (ICV) administration of TIP39. Ten male rats were surgically prepared with a guide cannula into the lateral ventricle and a catheter into the jugular vein. Each rat received ICV administration of 10 ug TIP39 or 10 ul vehicle. Blood samples were taken from the jugular vein for GH and prolactin measurement every 15 min for three hours. TIP39 caused a significant suppression of GH secretion, in terms of the total area under the curve, peak area and peak amplitude, as compared to vehicle injection. TIP39 had no effect on plasma prolactin levels. These data demonstrate that ICV administration of TIP39 exerts a specific inhibitory action on spontaneous GH secretion. TIP39 may be an important negative regulator of GH neuroendocrine axis.

L17 ANSWER 28 OF 44

MEDLINE on STN

DUPLICATE 9

ACCESSION NUMBER: 1999427840 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10499494

TITLE: Comparison of rat and human parathyroid hormone 2 (PTH2) receptor activation: PTH is a low potency partial agonist at the rat PTH2 receptor.

AUTHOR: Hoare S R; Bonner T I; Usdin T B

CORPORATE SOURCE: Unit on Cell Biology, Laboratory of Genetics, National Institute of Mental Health, Bethesda, Maryland 20892-4094, USA.

SOURCE: Endocrinology, (1999 Oct) Vol. 140, No. 10, pp. 4419-25. Journal code: 0375040. ISSN: 0013-7227.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 199910

ENTRY DATE: Entered STN: 26 Oct 1999

Last Updated on STN: 26 Oct 1999

Entered Medline: 12 Oct 1999

AB The human PTH2 receptor, expressed in tissue culture cells, is selectively activated by PTH. Detailed investigation of its anatomical and cellular distribution has been performed in the rat. It is expressed by neurons in a number of brain nuclei; by endocrine cells that include pancreatic islet somatostatin cells, thyroid parafollicular cells, and peptide secreting cells in the gastrointestinal tract; and by cells in the vasculature and heart. The physiological role of the PTH2 receptor expressed by these cells remains to be determined. All pharmacological studies performed to date have used the human receptor. We have now isolated a complementary DNA including the entire coding sequence of the rat PTH2 receptor and compared its pharmacological profile with that of the human PTH2 receptor when each is expressed in COS-7 cells. PTH-based peptides, including rat PTH(1-84), rat PTH(1-34), and human PTH(1-34), have low potency at the rat PTH2 receptor for stimulation of adenylyl cyclase (EC50 = 19-140 nM). When compared with the effect of a bovine hypothalamic extract, PTH-based peptides are partial agonists at the rat PTH2 receptor. This suggests that PTH is unlikely to be a physiologically important endogenous ligand



for the PTH2 receptor. A peptide homologous to an activity detected in a bovine hypothalamic extract is a good candidate for the endogenous PTH2 receptor ligand.

L17 ANSWER 29 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:456353 BIOSIS  
DOCUMENT NUMBER: PREV199900456353  
TITLE: Direct identification of two contact sites for parathyroid hormone (PTH) in the novel PTH-2 receptor using photoaffinity cross-linking.  
AUTHOR(S): Behar, Vered; Bisello, Alessandro; Rosenblatt, Michael; Chorev, Michael [Reprint author]  
CORPORATE SOURCE: Division of Bone and Mineral Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Avenue (HIM 944), Boston, MA, 02215, USA  
SOURCE: Endocrinology, (Sept., 1999) Vol. 140, No. 9, pp. 4251-4261. print.  
CODEN: ENDOAO. ISSN: 0013-7227.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 1 Nov 1999  
Last Updated on STN: 3 May 2000

AB Direct examination of the interacting sites between PTH and the human PTH2 receptor (PTH2R) was conducted by photoaffinity cross-linking followed by protein digestion and mapping of the radiolabeled photoconjugated receptor. Photoreactive analogs of PTH, individually substituted with an L-p-benzoylphenylalanine (Bpa) at each of the first 6 N-terminal positions, were pharmacologically evaluated in cells stably expressing recombinant PTH2R. One highly bioactive analog, (Bpa1,Nle8,18,Arg13,26,27,L-2-Nal23,Tyr34)PTH-(1-34)NH2 (Bpa1-PTH), was chosen for cross-linking studies. In addition, a PTH analog in which the photoreactive moiety is at the mid-region position 13 (K13) was demonstrated to be bioactive, then cross-linked to PTH2R. The minimal digestion-restricted domain containing the contact site ("contact domain") for 125I-Bpa1-PTH is in the sixth transmembrane domain and part of the third extracellular loop, spanning residues Ser364-Met395 of the receptor. This domain was further confirmed and refined by cross-linking 125I-Bpa1-PTH to two receptor mutants, PTH2R(V380M)- and PTH2R(V380M,M395L)-receptors. Treatment of the cross-linked conjugates with cyanogen bromide identified a single amino acid (position 380) as the putative contact point. The contact domain for 125I-K13 is located in the N-terminal extracellular tail of the receptor (in the C-terminal portion) and spans Gln138-Met147. Further validation of this contact domain was accomplished by photocross-linking to point-mutated PTH2R(K137R) receptor. Previous studies in which PTH analogs were cross-linked to human PTH/PTHrP receptor (PTH1R) identified Met425 and Phe173-Met189 as the contact sites for Bpa1-PTH and K13, respectively. These studies demonstrate that both receptor subtypes, PTH1- and PTH2-receptors, use analogous sites for interaction with positions 1 and 13 in PTH.

L17 ANSWER 30 OF 44 MEDLINE on STN DUPLICATE 10

ACCESSION NUMBER: 1999312160 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 10385434  
TITLE: Distribution of the parathyroid hormone 2 receptor in rat: immunolocalization reveals expression by several endocrine cells.  
AUTHOR: Usdin T B; Hilton J; Vertesi T; Harta G; Segre G; Mezey E  
CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892-4094, USA.. usdin@codon.nih.gov  
CONTRACT NUMBER: DK-47237 (NIDDK)  
SOURCE: Endocrinology, (1999 Jul) Vol. 140, No. 7, pp. 3363-71.  
Journal code: 0375040. ISSN: 0013-7227.

PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 199907  
ENTRY DATE: Entered STN: 27 Jul 1999  
Last Updated on STN: 27 Jul 1999  
Entered Medline: 15 Jul 1999

AB The PTH2 receptor is a G protein-coupled receptor selectively activated by PTH. We are studying the receptors distribution to guide the investigation of its physiological function. We have now generated an antibody from a C-terminal peptide sequence of the PTH2 receptor and used this to study its cellular distribution. Labeling with the antibody identified a number of endocrine cells expressing the PTH2 receptor, including thyroid parafollicular cells, pancreatic islet D cells, and some gastrointestinal peptide synthesizing cells. There was complete overlap of PTH2 receptor labeling with somatostatin in pancreatic islets, and partial overlap with somatostatin in thyroid parafollicular cells and in the gastrointestinal tract. Furthermore, observations made previously by in situ hybridization histochemistry, including expression throughout the cardiovascular system, as well as by discrete populations of cells within the gastrointestinal tract and reproductive system were confirmed. These data suggest a broad role for the PTH2 receptor, especially within the endocrine system, and provide a basis for experimental exploration of its physiology.

L17 ANSWER 31 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:148799 BIOSIS  
DOCUMENT NUMBER: PREV200000148799  
TITLE: Anatomical evidence that parathyroid hormone 2 receptor is involved in modulation of nociception.  
AUTHOR(S): Wang, T. [Reprint author]; Mezey, E.; Palkovits, M. [Reprint author]; Usdin, T. B. [Reprint author]  
CORPORATE SOURCE: Lab of Genetics, NIMH, Bethesda, MD, 20892, USA  
SOURCE: Society for Neuroscience Abstracts, (1999) Vol. 25, No. 1-2, pp. 2221. print.  
Meeting Info.: 29th Annual Meeting of the Society for Neuroscience. Miami Beach, Florida, USA. October 23-28, 1999. Society for Neuroscience.  
ISSN: 0190-5295.  
DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
LANGUAGE: English  
ENTRY DATE: Entered STN: 19 Apr 2000  
Last Updated on STN: 4 Jan 2002

L17 ANSWER 32 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 11

ACCESSION NUMBER: 1998:134086 BIOSIS  
DOCUMENT NUMBER: PREV199800134086  
TITLE: Transmembrane residues together with the amino terminus limit the response of the parathyroid hormone (PTH) 2 receptor to PTH-related peptide.  
AUTHOR(S): Turner, Paul R. [Reprint author]; Mefford, Suzanne; Bambino, Tom; Nissenson, Robert A.  
CORPORATE SOURCE: Endocrine Unit, VAMC, 4150 Clement St., San Francisco, CA 94121, USA  
SOURCE: Journal of Biological Chemistry, (Feb. 13, 1998) Vol. 273, No. 7, pp. 3830-3837. print.  
CODEN: JBCHA3. ISSN: 0021-9258.  
DOCUMENT TYPE: Article  
LANGUAGE: English

ENTRY DATE: Entered STN: 20 Mar 1998  
Last Updated on STN: 4 May 1998

AB The mechanisms of ligand binding and receptor activation for G-protein-coupled receptors in the secretin/parathyroid hormone (PTH) receptor subfamily are not understood. The PTH1 receptor (PTH1R) signals in response to both PTH and parathyroid hormone-related peptide (PTHrP), whereas the PTH2 receptor (PTH2R) responds only to PTH, not to PTHrP. To locate PTHrP discriminatory domains in the PTH2R, we generated PTH1R/PTH2R chimeras in which the extracellular amino-terminal domains were exchanged. Production of cAMP in response to 1  $\mu$ M PTHrP or PTH was identical in cells expressing the PTH1R with the PTH2R amino terminus and in cells expressing the PTH2R with the PTH1R amino terminus. The ability of the chimeric receptor with the PTH2R amino terminus to respond fully to PTHrP showed that the body of the PTH2R must contain sites that limit the response to PTHrP. Mutations to PTH1R sequence were therefore made in each of the seven transmembrane domains of the PTH2R. Mutations in transmembrane domains 3 and 7 resulted in receptors able to respond to PTHrP. Thus, residues in more than one domain form a barrier or filter, allowing the receptor to discriminate between different ligands.

L17 ANSWER 33 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:28099 BIOSIS

DOCUMENT NUMBER: PREV199800028099

TITLE: Residues in the membrane-spanning and extracellular loop regions of the parathyroid hormone (PTH)-2 receptor determine signaling selectivity for PTH and PTH-related peptide.

AUTHOR(S): Bergwitz, Clemens; Jusseaume, Scott A.; Luck, Michael D.; Jueppner, Harald; Gardella, Thomas J. [Reprint author]

CORPORATE SOURCE: Dep. Med., Children's Service, Endocrine Unit, Massachusetts General Hosp. Harvard Med. Sch., Boston, MA 02114, USA

SOURCE: Journal of Biological Chemistry, (Nov. 14, 1997) Vol. 272, No. 46, pp. 28861-28868. print.  
CODEN: JBCHA3. ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 14 Jan 1998  
Last Updated on STN: 24 Feb 1998

AB The parathyroid hormone (PTH)-2 receptor displays strong ligand selectivity in that it responds fully to PTH but not at all to PTH-related peptide (PTHrP). In contrast, the PTH-1 receptor (PTH/PTHrP receptor) responds fully to both ligands. Previously it was shown that two divergent residues in PTH and PTHrP account for PTH-2 receptor selectivity; position 23 (Trp in PTH and Phe in PTHrP) determines binding selectivity and position 5 (Ile in PTH and His in PTHrP) determines signaling selectivity. To identify sites in the PTH-2 receptor involved in discriminating between His5 and Ile5, we constructed PTH-2 receptor/PTH-1 receptor chimeras, expressed them in COS-7 cells, and tested for cAMP responsiveness to (Trp23) PTHrP-(1-36), and to the nondiscriminating peptide (Ile5,Trp23)PTHrP-(1-36) (the Phe23 fwdarw Trp modification enabled high affinity binding of each ligand to the PTH-2 receptor). The chimeras revealed that the membrane-spanning/loop region of the receptor determined His5/Ile5 signaling selectivity. Subsequent analysis of smaller cassette substitutions and then individual point mutations led to the identification of two single residues that function as major determinants of residue 5 signaling selectivity. These residues, Ile244 at the extracellular end of transmembrane helix 3, and Tyr318 at the COOH-terminal portion of extracellular loop 2, are replaced by Leu and Ile in the PTH-1 receptor, respectively. The results thus indicate a functional interaction between two residues in the core region of the PTH-2 receptor and residue 5 of the ligand.

L17 ANSWER 34 OF 44 MEDLINE on STN DUPLICATE 12

ACCESSION NUMBER: 97156632 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9003022

TITLE: Evidence for a parathyroid hormone-2 receptor selective ligand in the hypothalamus.

AUTHOR: Usdin T B

CORPORATE SOURCE: National Institute of Mental Health, Bethesda MD 20892, USA.. usdin@codon.nih.gov

SOURCE: Endocrinology, (1997 Feb) Vol. 138, No. 2, pp. 831-4. Journal code: 0375040. ISSN: 0013-7227.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals

ENTRY MONTH: 199702

ENTRY DATE: Entered STN: 5 Mar 1997  
Last Updated on STN: 5 Mar 1997  
Entered Medline: 18 Feb 1997

AB The PTH2 receptor is expressed in several brain nuclei but we have been unable to detect mRNA encoding PTH, which is the only known ligand for the PTH2 receptor, in the brain. We now have evidence for a PTH2 receptor selective ligand in an acid-acetone extract made from bovine hypothalamus. The partially purified extract activates the PTH2 receptor more effectively than it activates the PTH/PTHrP receptor, while PTH activates these two receptors at similar concentration. The activity appears immunologically distinct from PTH and its effect is potentially antagonized by [D-Trp12]bPTH(7-34). These data provide evidence for a biologically active peptide, which may be related to PTH, and which is a potential new neurotransmitter or hormone.

L17 ANSWER 35 OF 44 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:288536 SCISEARCH

THE GENUINE ARTICLE: WT133

TITLE: The parathyroid hormone-2 receptor: Current status

AUTHOR: Usdin T B

CORPORATE SOURCE: NIMH, GENET SECT, BETHESDA, MD 20892

COUNTRY OF AUTHOR: USA

SOURCE: EXPERIMENTAL AND MOLECULAR MEDICINE, (31 MAR 1997) Vol. 29, No. 1, pp. 13-17. ISSN: 1226-3613.

PUBLISHER: KOREAN SOC MED BIOCHEMISTRY MOLECULAR BIOLOGY, #812 KOFST, 635-4 YOKSAM-DONG KANGNAM-GU, SEOUL 135-703, SOUTH KOREA.

DOCUMENT TYPE: General Review; Journal

LANGUAGE: English

REFERENCE COUNT: 32

ENTRY DATE: Entered STN: 1997  
Last Updated on STN: 1997

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB G-protein coupled receptors form a large superfamily of plasma membrane proteins which serve a variety of signal transduction roles. New receptors continue to be identified. Based on sequence homology the superfamily can currently be divided into three families, the rhodopsin family which includes the vast majority of identified receptors, and the secretin. and metabotropic glutamate receptor families which share a general architecture with each other and the rhodopsin family but no obvious sequence identity. Screening for additional members of the secretin family led to the identification of the parathyroid hormone-2 (PTH2) receptor. Ligand recognition by the PTH2 receptor partially overlaps that of the PTH/parathyroid hormone-related peptide (PTHrP) receptor. This has facilitated structure-function analysis of ligands for these receptors. The physiological role of the PTH2 receptor is under investigation but its



distribution suggests that it may be a neurotransmitter receptor and could participate in modulation of a number of organ systems. The relative abundance of PTH2 receptor mRNA in the brain and the inability to detect mRNA encoding PTH, its only currently identified ligand, suggest the existence of another endogenous ligand, for which evidence has recently been obtained.

L17 ANSWER 36 OF 44 MEDLINE on STN DUPLICATE 13  
ACCESSION NUMBER: 96426194 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 8828488  
TITLE: Distribution of parathyroid hormone-  
2 receptor messenger ribonucleic acid in rat.  
AUTHOR: Usdin T B; Bonner T I; Harta G; Mezey E  
CORPORATE SOURCE: Laboratory of Cell Biology, National Institute of Mental  
Health, National Institutes of Health, Bethesda, Maryland  
20892, USA.. usdin@codon.nih.gov  
SOURCE: Endocrinology, (1996 Oct) Vol. 137, No. 10, pp. 4285-97.  
Journal code: 0375040. ISSN: 0013-7227.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
OTHER SOURCE: GENBANK-L19475; GENBANK-U55836  
ENTRY MONTH: 199612  
ENTRY DATE: Entered STN: 28 Jan 1997  
Last Updated on STN: 28 Jan 1997  
Entered Medline: 18 Dec 1996

AB The PTH2 receptor is a recently identified G protein-coupled receptor activated by PTH. Its amino acid sequence is most similar to the PTH/PTHrP receptor, but unlike the PTH/PTHrP receptor, it is activated by PTH and not by PTH-related peptide. We previously demonstrated using Northern blots that expression of PTH2 receptor messenger RNA was greatest within the brain and occurred at lower levels in pancreas, testis, and placenta. We have now obtained a complementary DNA encoding the rat PTH2 receptor and used it to study the distribution of the PTH2 receptor using in situ hybridization histochemistry. PTH2 receptor messenger RNA is abundantly expressed in arterial and cardiac endothelium and at lower levels in vascular smooth muscle. It is also abundant in the lung, both within bronchi and in the parenchyma, and is present within the exocrine pancreas. It is expressed by sperm in the head of the epididymis. A small number of cells associated with the vascular pole of renal glomeruli express the receptor. These data suggest that the PTH2 receptor may be responsible for PTH effects in a number of physiological systems.

L17 ANSWER 37 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
ACCESSION NUMBER: 1996:503518 BIOSIS  
DOCUMENT NUMBER: PREV199699225874  
TITLE: Histidine at position 5 is the specificity "switch" between two parathyroid hormone receptor subtypes.  
AUTHOR(S): Behar, Vered; Nakamoto, Chizu; Greenberg, Zvi; Bisello, Alessandro; Suva, Larry J.; Rosenblatt, Michael; Chorev, Michael [Reprint author]  
CORPORATE SOURCE: Div. Bone Mineral Metabolism, Beth Israel Hosp., 330 Brooklin Ave., Boston, MA 02215, USA  
SOURCE: Endocrinology, (1996) Vol. 137, No. 10, pp. 4217-4224.  
CODEN: ENDOAO. ISSN: 0013-7227.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 14 Nov 1996  
Last Updated on STN: 14 Nov 1996

AB The PTH and PTH-related protein (PTHrP) system consists of two hormones, at least two G protein-coupled seven-transmembrane domain receptors, and

at least two intracellular signal transduction pathways for each receptor. The PTH/PTHrP receptor is present in the conventional target tissues of PTH action, namely kidney and bone. Both PTH and PTHrP bind to and activate the PTH/PTHrP receptor with equal affinity and efficacy. The newly discovered receptor, termed the human (h) PTH2 receptor, has 70% homology with the PTH/PTHrP receptor, but is found predominantly in brain and pancreas. It interacts selectively with PTH and not with PTHrP. PTH and PTHrP differ in several positions, including position 5 (Ile in PTH; His in PTHrP). To define the role of position 5 in receptor selectivity, we designed and synthesized a series of hybrid analogs containing specific elements of both the PTH and PTHrP sequences. Using human cell lines stably expressing either human receptor subtype, we evaluated the biological profile of the hybrids in assays of receptor binding and action. Both point-mutated hybrids, (Ile-5)PTH-(1-34) and (His-5)PTH-(1-34), bind to and stimulate cAMP accumulation and the release of cytosolic free calcium in HEK293/C-21, a clonal human embryonic kidney cell line stably expressing the recombinant hPTH/ PTHrP receptor. However, only (Ile-5)PTHrP-(1-34), and not (His-5)PTH-(1-34), binds to and stimulates cAMP accumulation and the release of cytosolic free calcium in HEK293/BP-16, a clonal human embryonic kidney cell line stably expressing the recombinant hPTH2 receptor. The segmental hybrid PTHrP-(1-14)-PTH-(15-34) binds to and activates the hPTH/PTHrP receptor, but not the hPTH2 receptor, similar to the biological profile of His-5-containing ligands: PTHrP(1-34) and (His-5)PTH-(1-34). Exchanging Ile-5 for His-5 in the segmental hybrid produces the analog (Ile-5)PTHrP-(1-14)-PTH-(15-34), which interacts with both receptor subtypes. We conclude that His-5 in PTHrP is the major structural determinant of receptor subtype specificity in the hPTH/PTHrP and hPTH2 two-receptor system. The mechanism of the specificity "switch" remains to be elucidated, but may result from a subtle perturbation of the bioactive conformation and/or from a direct steric hindrance at the hPTH2 receptor-ligand interface created by histidine at position 5. The hPTH2, but not the hPTH/PTHrP, receptor can discriminate between the two hormones based on the structural differences generated at position 5.

L17 ANSWER 38 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1995:155927 BIOSIS  
DOCUMENT NUMBER: PREV199598170227  
TITLE: Acute effect of parathyroid hormone on urine concentration in the rat.  
AUTHOR(S): Carney, S. L. [Reprint author]; Gillies, A. H. B.  
CORPORATE SOURCE: Dep. Med., John Hunter Hosp., Locked Bag 1, Hunter Region Mail Cent., NSW 2310, Australia  
SOURCE: Clinical Science (London), (1995) Vol. 88, No. 2, pp. 197-201.  
CODEN: CSCIAE. ISSN: 0143-5221.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 11 Apr 1995  
Last Updated on STN: 12 Apr 1995

AB 1. It has been demonstrated that parathyroid hormone can increase adenylate cyclase activity in the rat papilla, produce a small antidiuretic effect and in vitro can interfere with the action of arginine vasopressin on water transport. Clearance studies were performed in the anaesthetized water diuretic thyroparathyroidectomized rat to evaluate further the effect of parathyroid hormone on urine concentration in the presence and absence of arginine vasopressin. 2. A maximal phosphaturic concentration of rat parathyroid hormone (2  $\mu$ -g/kg) reduced urine flow from 125  $\pm$  7 to 81  $\pm$  9  $\mu$ -l/kg) within 10 min (P lt 0.01). Addition of a maximal antidiuretic concentration of arginine vasopressin (100 ng/kg) produced a delayed and diminished antidiuretic response when compared with a group of rats not pretreated with parathyroid hormone (47  $\pm$  5 compared with 27  $\pm$  5  $\mu$ -l/min; P lt

0.01). However, a supramaximal arginine vasopressin concentration (1000 ng/kg) produced a maximal antidiuretic effect in the presence of parathyroid hormone. 3. To evaluate further the inhibitory effect of para-thyroid hormone on arginine vasopressin-induced antidiuresis, parathyroid hormone (2  $\mu$ -g/kg) was administered to one group of rats and a minimally effective arginine vasopressin concentration (7.5 ng/kg) to another group, which produced a similar antidiuretic effect. However, the subsequent effect of a maximal antidiuretic arginine vasopressin concentration (100 ng/kg) was again significantly blunted in the group pretreated with parathyroid hormone. 4. Parathyroid hormone produced only a small increase in mean plasma calcium concentration, and glomerular filtration rate was not altered by either hormone. 5. These results demonstrate that high physiological concentrations of parathyroid hormone do have a significant antidiuretic effect and can interfere with the action of arginine vasopressin. This suggests that parathyroid hormone may act as a partial agonist to arginine vasopressin in the collecting system.

L17 ANSWER 39 OF 44 MEDLINE on STN DUPLICATE 14  
 ACCESSION NUMBER: 94353932 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8073873  
 TITLE: Hormone-dependent enhancement in binding of oto- and nephrotoxic aminoglycoside antibiotics.  
 AUTHOR: Iwamori M; Tayama N; Nomura Y; Nagai Y  
 CORPORATE SOURCE: Department of Biochemistry, Faculty of Medicine, University of Tokyo, Japan.  
 SOURCE: Acta oto-laryngologica. Supplementum, (1994) Vol. 514, pp. 117-21.  
 Journal code: 0370355. ISSN: 0365-5237.  
 PUB. COUNTRY: Norway  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199409  
 ENTRY DATE: Entered STN: 6 Oct 1994  
 Last Updated on STN: 3 Mar 2000  
 Entered Medline: 23 Sep 1994

AB Intraperitoneal administration of gentamicin (40 mg/ml) to the guinea pig is known to cause damage of the tissue of the kidneys and auditory organs. By indirect immunohistochemical staining with anti-gentamicin antiserum, those cells with high affinity to gentamicin in the auditory organs and kidneys were the hair cells in the cochlea and the epithelial cells in the renal tubules. The concentrations of gentamicin in the serum and perilymph of the guinea pig with tissue damage were found to be 2 and 0.6 mg/ml at the maximal levels, respectively, by high performance liquid chromatography. The same concentration of gentamicin, 2 mg/ml, also inhibited cell growth and resulted in cell damage of the renal tubule-derived cell lines, JTC-12 and MDCK. Among the antibiotics examined, i.e. streptomycin, gentamicin, fradiomycin and kanamycin, gentamicin showed the strongest effect on growth inhibition of the renal tubule-derived cells, when these cells were cultured in a medium with 5% fetal calf serum. Although the number of JTC-12 cells in the medium without fetal calf serum remained the same for a week, even with the addition of either gentamicin (0.5 mg/ml) or parathyroid hormone (2 mM) the coadministration of gentamicin and parathyroid hormone resulted in a loss of cells due to cellular death, and the amount of gentamicin bound onto the cells cultured with gentamicin plus parathyroid hormone was significantly higher than that with gentamicin alone. These results indicate that the expression of the receptor for gentamicin on the cell surface is greatly enhanced by hormonal stimulation.

L17 ANSWER 40 OF 44 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1994:419801 SCISEARCH  
THE GENUINE ARTICLE: NU455  
TITLE: HORMONE-DEPENDENT ENHANCEMENT IN BINDING OF OTOTOXIC AND  
NEPHROTOXIC AMINOGLYCOSIDE ANTIBIOTICS  
AUTHOR: IWAMORI M (Reprint); TAYAMA N; NOMURA Y; NAGAI Y  
CORPORATE SOURCE: UNIV TOKYO, FAC MED, DEPT BIOCHEM, TOKYO 113, JAPAN  
(Reprint); UNIV TOKYO, FAC MED, DEPT OTOLARYNGOL, TOKYO  
113, JAPAN; SHOWA UNIV, SCH MED, DEPT OTORHINOLARYNGOL,  
TOKYO 142, JAPAN; TOKYO METROPOLITAN INST MED SCI, TOKYO  
113, JAPAN  
COUNTRY OF AUTHOR: JAPAN  
SOURCE: ACTA OTO-LARYNGOLOGICA, (1994) Supp. [514], pp. 117-121.  
ISSN: 0001-6489.  
PUBLISHER: SCANDINAVIAN UNIVERSITY PRESS, PO BOX 2959 TOYEN, JOURNAL  
DIVISION CUSTOMER SERVICE, N-0608 OSLO, NORWAY.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE; CLIN  
LANGUAGE: English  
REFERENCE COUNT: 13  
ENTRY DATE: Entered STN: 1994  
Last Updated on STN: 1994

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Intraperitoneal administration of gentamicin (40 mg/ml) to the guinea pig is known to cause damage of the tissue of the kidneys and auditory organs. By indirect immunohistochemical staining with anti-gentamicin antiserum, those cells with high affinity to gentamicin in the auditory organs and kidneys were the hair cells in the cochlea and the epithelial cells in the renal tubules. The concentrations of gentamicin in the serum and perilymph of the guinea pig with tissue damage were found to be 2 and 0.6 mg/ml at the maximal levels, respectively, by high performance liquid chromatography. The same concentration of gentamicin, 2 mg/ml, also inhibited cell growth and resulted in cell damage of the renal tubule-derived cell lines, JTC-12 and MDCK. Among the antibiotics examined, i.e. streptomycin, gentamicin, fradiomycin and kanamycin, gentamicin showed the strongest effect on growth inhibition of the renal tubule-derived cells, when these cells were cultured in a medium with 5% fetal calf serum. Although the number of JTC-12 cells in the medium without fetal calf serum remained the same for a week, even with the addition of either gentamicin (0.5 mg/ml) or parathyroid hormone (2 mM) the coadministration of gentamicin and parathyroid hormone resulted in a loss of cells due to cellular death, and the amount of gentamicin bound onto the cells cultured with gentamicin plus parathyroid hormone was significantly higher than that with gentamicin alone. These results indicate that the expression of the receptor for gentamicin on the cell surface is greatly enhanced by hormonal stimulation.

L17 ANSWER 41 OF 44 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1981:156408 BIOSIS  
DOCUMENT NUMBER: PREV198171026400; BA71:26400  
TITLE: ANALOGS OF PARATHYROID HORMONE CONTAINING D AMINO-ACIDS  
EVALUATION OF BIOLOGICAL ACTIVITY AND STABILITY.  
AUTHOR(S): COLTRERA M [Reprint author]; ROSENBLATT M; POTTS J T JR  
CORPORATE SOURCE: DEP MED, HARV MED SCH, BOSTON, MASS 02114, USA  
SOURCE: Biochemistry, (1980) Vol. 19, No. 18, pp. 4380-4385.  
CODEN: BICHAW. ISSN: 0006-2960.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH

AB Four analogs of parathyroid hormone (PTH) containing D-amino acids were synthesized. Substitutions were made within the fully biologically active fragment of PTH in the amino-terminal region, at position 2, and at the carboxyl terminus, at position 34. The carboxyl-terminal region contains



structural determinants important to receptor binding. The amino-terminal region plays a critical role in the hormone-stimulated activation of adenylate cyclase in vitro and the expression of hormonal activity in vivo. Placement of a D-amino acid at the carboxyl terminus yielded an analog, [D-Tyr34]b[bovine]PTH-(1-34)-amide, 270% as active in the in vitro renal adenylate cyclase assay as unsubstituted bPTH-(1-34). In contrast, placement of a D-amino acid in the amino-terminal region, as in analogs [D-Val2,D-Tyr34]-bPTH-(1-34)-amide and [D-Val2,D-Tyr34]bPTH-(2-34)-amide, resulted in nearly a complete loss of in vitro biological activity. Deletion of a single residue at the amino terminus, as in the analogue [D-Tyr34]bPTH-(2-34)-amide, also caused nearly total loss of biopotency. These marked declines in potency occurred despite the presence of activity-enhancing modifications at the carboxyl terminus of the latter 3 analogs. The most potent of the analogs, [D-Tyr34]-bPTH-(1-34)-amide, sustained an apparently spontaneous and complete loss of biopotency over several weeks. Detailed studies of the mechanism of inactivation revealed an unusual lability of methionine residues to oxidation. Reduction under controlled conditions restored nearly completely both the methionine content and biological activity. Placement of D-amino acids in the PTH sequence markedly alters hormonal activity: the change in bioactivity depends critically on the position selected for substitution. The observed instability of bioactivity also illustrates the need to either monitor biopotency of PTH and its analogs or utilize oxidation-stable hormone analogs in investigations of PTH mediated biological effects.

L17 ANSWER 42 OF 44 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1980:213199 HCAPLUS  
DOCUMENT NUMBER: 92:213199  
TITLE: Parathyroid hormone metabolism and electrolyte excretion in healthy controls and patients with nephrolithiasis  
AUTHOR(S): Dunzendorfer, U.; Schmidt-Gayk, H.  
CORPORATE SOURCE: Abt. Urol., Universitaetsklin. Frankfurt, Frankfurt/Main, Fed. Rep. Ger.  
SOURCE: Klinische Wochenschrift (1980), 58(3), 153-5  
CODEN: KLWOAZ; ISSN: 0023-2173  
DOCUMENT TYPE: Journal  
LANGUAGE: German

AB After injection of parathyroid hormone (2.5 IU/kg), the half-life of the hormone in serum was the same (30-40 min) in controls and in patients with idiopathic Ca oxalate renal calculi. They were also no differences between the 2 groups with respect to the effects of the hormone on urinary electrolyte excretion and on serum and urine cyclic AMP concns.

L17 ANSWER 43 OF 44 MEDLINE on STN

ACCESSION NUMBER: 79122133 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 217276  
TITLE: Antiphosphaturic action of 25 (OH) vitamin D3 in experimental Fanconi syndrome.  
AUTHOR: Popovtzer M M; Mehandru S K; Saghafi D; Blum M S  
SOURCE: The American journal of physiology, (1979 Feb) Vol. 236, No. 2, pp. E90-7.  
Journal code: 0370511. ISSN: 0002-9513.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197904  
ENTRY DATE: Entered STN: 15 Mar 1990  
Last Updated on STN: 15 Mar 1990  
Entered Medline: 26 Apr 1979

AB Renal handling of phosphorus was studied in the following groups of

parathyroidectomized rats with maleate-induced Fanconi syndrome: 1) 6 rats receiving intravenous parathyroid hormone, 2) 6 rats receiving intravenous dibutyryl cyclic AMP (DBcAMP), 3) 6 rats undergoing volume expansion with saline, 4) 12 rats receiving intravenous 25 (OH)vitamin D3, 5) 12 rats with acute hypercalcemia induced by intravenous CaCl2, 6) 6 rats with phosphate deprivation, and 7) 6 rats receiving intravenous calcitonin. Parathyroid hormone and calcitonin failed to increase the urinary excretion of both cAMP and phosphorus. Likewise, DBcAMP failed to increase the urinary excretion of phosphorus. Extracellular volume expansion and hypercalcemia (serum calcium 12.9 +/- 0.7 mg/100 ml) did not alter the tubular reabsorption of phosphorus. In phosphate-deprived animals, the fractional excretion 0.16 +/- 0.05 (mean +/- SE) was lower than that in the control animals (maleate-treated without phosphate depletion), 0.46 +/- 0.04 (P less than 0.001). 25 (OH)vitamin D3 decreased the fractional excretion of phosphorus from 0.39 +/- 0.03 in the control (maleate-treated not receiving 25 (OH)vitamin D3) to 0.23 +/- 0.02 (P less than 0.001) in the experimental animals. The present study demonstrated an antiphosphaturic effect of 25(OH)vitamin D3 in experimental Fanconi syndrome; the mechanism of this action is not well understood.

L17 ANSWER 44 OF 44 MEDLINE on STN  
 ACCESSION NUMBER: 76234606 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 181194  
 TITLE: Reversible resistance to the renal action of parathyroid hormone in man.  
 AUTHOR: Tomlinson S; Hendy G N; Pemberton D M; O'Riordan J L  
 SOURCE: Clinical science and molecular medicine, (1976 Jul) Vol. 51, No. 1, pp. 59-69.  
 Journal code: 0367540. ISSN: 0301-0538.  
 PUB. COUNTRY: ENGLAND: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 197609  
 ENTRY DATE: Entered STN: 13 Mar 1990  
 Last Updated on STN: 3 Feb 1997  
 Entered Medline: 25 Sep 1976

AB 1. Normal subjects showed a highly reproducible, rapid increase in plasma adenosine 3':5'-cyclic monophosphate (cyclic AMP) after an intravenous injection of 200 MRC units of highly purified bovine parathyroid hormone. 2. No significant increase in plasma cyclic AMP was observed after administration of bovine parathyroid hormone to patients with severe chronic renal failure. 3. Even when renal function was not impaired, some patients with primary hyperparathyroidism, who had high concentrations of endogenous parathyroid hormone, showed resistance to bovine parathyroid hormone and when this was injected intravenously it caused only a small increase in plasma cyclic AMP. This resistance was reversible since there was marked improvement in the response after parathyroidectomy, when endogenous parathyroid hormone concentration had fallen. 4. It was possible to reproduce this resistance to the hormone by intravenous infusion of bovine parathyroid hormone into normal subjects. When the hormone (1000 MRC units) was infused over 2 h, after an initial increase there was a progressive decline in plasma cyclic AMP concentration and a fall in urinary cyclic AMP excretion. The response to a standard test stimulus (200 MRC units of bovine parathyroid hormone given as a rapid intravenous injection) was examined at intervals after 1000 units of bovine parathyroid hormone had been infused. Initially, the response was severely impaired; at 4 h, partial recovery had occurred and, 24 h after the infusion, recovery of the response was complete. The resistance was therefore reversible. Infusion of the amino-terminal peptide, fragment 1-34, gave the same effect as infusion of intact hormone. Region-specific assays for the hormone were used to show

that the concentration of immuno-assayable hormone remained high during the infusions. 5. The mechanism of this reversible resistance to parathyroid hormone remains to be elucidated; it seems unlikely that circulating hormone fragments could account for the prolonged impairment in the responsiveness to the intact hormone. It is possible that alteration in the formation, intracellular degradation or, perhaps, release of cyclic AMP from the cells, is the cause. Changes in the characteristics of the hormone receptor sites might also explain the phenomenon.

=> his

HIS IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system. For a list of commands available to you in the current file, enter "HELP COMMANDS" at an arrow prompt (=>).

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

```
L1      2096 S MAPKAP##
L2      5 S L1 AND (MAPKAP-2 (W)KINASE?)
L3      4 DUP REM L2 (1 DUPLICATE REMOVED)
L4      119389 S PARATHYROID (W)HORMONE##
L5      6 S L1 AND L4
L6      5 DUP REM L5 (1 DUPLICATE REMOVED)
L7      221 S PARATHYROID (W)HORMONE-2
L8      1 S L1 AND L7
L9      68 S IMMUN? AND L7
L10     1 S L1 AND L9
L11     369433 S SKELETAL (W)MUSCLE
L12     387 S L4 AND L11
L13     0 S L7 AND L11
L14     76 S L7 AND (ISOLAT? OR PURIF?)
L15     33 DUP REM L14 (43 DUPLICATES REMOVED)
L16     87 S L7 AND (EXPRESS? OR EXCRET?)
L17     44 DUP REM L16 (43 DUPLICATES REMOVED)
```

=> s 17 (2w)ligand?

```
L18     12 L7 (2W) LIGAND?
```

=> dup rem l18

PROCESSING COMPLETED FOR L18

```
L19     4 DUP REM L18 (8 DUPLICATES REMOVED)
```

=> d 1-4 ibibab

'IBIBAB' IS NOT A VALID FORMAT

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):'end

'END' IS NOT A VALID FORMAT

In a multifile environment, a format can only be used if it is valid in at least one of the files. Refer to file specific help messages or the STNGUIDE file for information on formats available in individual files.

REENTER DISPLAY FORMAT FOR ALL FILES (FILEDEFAULT):end

=> d 1-4 ibib ab

L19 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2004353940 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15257146  
 TITLE: Tuberoinfundibular peptide of 39 residues decreases pain-related affective behavior.  
 AUTHOR: LaBuda Christopher J; Usdin Ted B  
 CORPORATE SOURCE: Laboratory of Genetics, National Institute of Mental Health, Bldg. 36/Rm. 3D06, 36 Convent Dr. MSC 4094, Bethesda, MD 20892-4094, USA.  
 SOURCE: Neuroreport, (2004 Aug 6) Vol. 15, No. 11, pp. 1779-82. Journal code: 9100935. ISSN: 0959-4965.  
 PUB. COUNTRY: England: United Kingdom  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200410  
 ENTRY DATE: Entered STN: 17 Jul 2004  
 Last Updated on STN: 22 Oct 2004  
 Entered Medline: 21 Oct 2004  
 AB Tuberoinfundibular peptide of 39 residues (TIP39) is a recently identified parathyroid hormone 2 receptor ligand  
 . Their CNS distributions suggest potential involvement in neuroendocrine, limbic and sensory processing functions. Herein we investigate the analgesic and antinociceptive actions of brain delivery of TIP39 in adult male rats. Intracerebroventricular (i.c.v.) TIP39 did not change hot-plate paw withdrawal latency or formalin test behavioral responses. TIP39 partially reversed tactile withdrawal hypersensitivity following carageenan administration. In the place/escape avoidance paradigm (PEAP), which evaluates affective components of responses to noxious stimuli by presenting a choice between a naturally preferred environment paired with stimulation of a carrageenan sensitized paw and a less preferred environment paired with stimulation of a less sensitive paw, TIP39 decreased the apparent aversiveness of sensitive paw stimulation. Because acute sensory thresholds were unaffected by TIP39, and the effects of i.c.v. TIP39 were opposite in direction from previously described effects of intrathecal TIP39, this suggests that TIP39 may modulate an affective component of nociception within the brain.

L19 ANSWER 2 OF 4 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
 ACCESSION NUMBER: 2004:205393 BIOSIS  
 DOCUMENT NUMBER: PREV200400205920  
 TITLE: The localization and projections of TIP39 neurons suggest limbic, endocrine, auditory and nociceptive functions of the neuropeptide.  
 AUTHOR(S): Dobolyi, A. [Reprint Author]; Palkovits, M. [Reprint Author]; Bodnar, I. [Reprint Author]; Usdin, T. B. [Reprint Author]  
 CORPORATE SOURCE: Lab. of Genet., Natl. Inst. of Mental Hlth., NIH, Bethesda, MD, USA  
 SOURCE: Society for Neuroscience Abstract Viewer and Itinerary Planner, (2003) Vol. 2003, pp. Abstract No. 889.23. <http://sfn.scholarone.com>. e-file. Meeting Info.: 33rd Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 08-12, 2003. Society of Neuroscience.  
 DOCUMENT TYPE: Conference; (Meeting)  
 Conference; Abstract; (Meeting Abstract)  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 14 Apr 2004  
 Last Updated on STN: 14 Apr 2004  
 AB Tuberoinfundibular peptide of 39 residues (TIP39) was recently purified as a parathyroid hormone 2 receptor ligand. Our previous in situ hybridization and RT-PCR studies showed that TIP39 neurons are restricted to two distinct nervous system



areas, one at the diencephalon-midbrain junction, and the other in the lateral pons. We have now investigated the precise localization and the projections of TIP39 neurons. One group of TIP39 cells extends from the caudal hypothalamus to the mesencephalic central gray. Most cells are near the midline, in the subparafascicular nucleus and medial to it and to the fasciculus retroflexus. Lesions here caused the disappearance of TIP39 fibers ipsilaterally in the limbic cortex, nucleus accumbens, lateral septum, bed nucleus of the stria terminalis, amygdaloid nuclei, thalamic paraventricular nucleus, and hypothalamic paraventricular, dorsomedial and arcuate nuclei. TIP39 terminals surround cell bodies and proximal dendrites of CRH cells in the paraventricular nucleus and are closely apposed to some GHRH and somatostatin cells. TIP39 cells in the lateral pons occupy an area between the pontine reticular nucleus and the nuclei of the lateral lemniscus, immediately dorsal to the rubrospinal tract in the caudal part of the medial paralemniscal nucleus. Lesions here cause disappearance of TIP39 fibers ipsilaterally from the medial geniculate body, periaqueductal central gray, deep nuclei of the superior colliculus, inferior colliculus, nuclei of the lateral lemniscus, lateral parabrachial nucleus, locus coeruleus, trapezoid body, periolivary nuclei, and spinal cord. Our results suggest that the diencephalic TIP39 participates in limbic and endocrine functions, and that TIP39 in the lateral pons defines a new nucleus with previously undescribed auditory and nociceptive projections.

L19 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN  
 ACCESSION NUMBER: 1999:85708 BIOSIS  
 DOCUMENT NUMBER: PREV199900085708  
 TITLE: Progress on the identification of a novel PTH2  
 receptor-selective peptide from the hypothalamus.  
 AUTHOR(S): Usdin, T. B. [Reprint author]; Baptiste, L.; Jaffe, H.  
 CORPORATE SOURCE: Sect. Genetics, NIMH, Bethesda, MD 20892, USA  
 SOURCE: Society for Neuroscience Abstracts, (1998) Vol. 24, No.  
 1-2, pp. 2044. print.  
 Meeting Info.: 28th Annual Meeting of the Society for  
 Neuroscience, Part 2. Los Angeles, California, USA.  
 November 7-12, 1998. Society for Neuroscience.  
 ISSN: 0190-5295.  
 DOCUMENT TYPE: Conference; (Meeting)  
 Conference; Abstract; (Meeting Abstract)  
 Conference; (Meeting Poster)  
 LANGUAGE: English  
 ENTRY DATE: Entered STN: 1 Mar 1999  
 Last Updated on STN: 1 Mar 1999

L19 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 2  
 ACCESSION NUMBER: 97156632 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 9003022  
 TITLE: Evidence for a parathyroid hormone-  
 2 receptor selective ligand in the  
 hypothalamus.  
 AUTHOR: Usdin T B  
 CORPORATE SOURCE: National Institute of Mental Health, Bethesda MD 20892,  
 USA.. usdin@codon.nih.gov  
 SOURCE: Endocrinology, (1997 Feb) Vol. 138, No. 2, pp. 831-4.  
 Journal code: 0375040. ISSN: 0013-7227.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
 ENTRY MONTH: 199702  
 ENTRY DATE: Entered STN: 5 Mar 1997  
 Last Updated on STN: 5 Mar 1997  
 Entered Medline: 18 Feb 1997

AB The PTH2 receptor is expressed in several brain nuclei but we have been

unable to detect mRNA encoding PTH, which is the only known ligand for the PTH2 receptor, in the brain. We now have evidence for a PTH2 receptor selective ligand in an acid-acetone extract made from bovine hypothalamus. The partially purified extract activates the PTH2 receptor more effectively than it activates the PTH/PTHrP receptor, while PTH activates these two receptors at similar concentration. The activity appears immunologically distinct from PTH and its effect is potently antagonized by [D-Trp12]bPTH(7-34). These data provide evidence for a biologically active peptide, which may be related to PTH, and which is a potential new neurotransmitter or hormone.

=> d his

(FILE 'HOME' ENTERED AT 14:39:29 ON 31 JUL 2006)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 14:39:58 ON 31 JUL 2006

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L1      2096 S MAPKAP##
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L16     87 S L7 AND (EXPRESS? OR EXCRET?)
L17     44 DUP REM L16 (43 DUPLICATES REMOVED)
L18     12 S L7 (2W)LIGAND?
L19     4 DUP REM L18 (8 DUPLICATES REMOVED)
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L17     44 DUP REM L16 (43 DUPLICATES REMOVED)
L18     12 S L7 (2W)LIGAND?
L19     4 DUP REM L18 (8 DUPLICATES REMOVED)
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L7 ANSWER 201 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1995:460422 HCAPLUS

DOCUMENT NUMBER: 122:205722

TITLE: Acute effect of parathyroid hormone on urine concentration in the rat

AUTHOR(S): Carney, S. L.; Gillies, A. H. B.

CORPORATE SOURCE: Faculty Medicine, University Newcastle, Newcastle, Australia

SOURCE: Clinical Science (1995), 88(2), 197-201

CODEN: CSCIAE; ISSN: 0143-5221

DOCUMENT TYPE: Journal

LANGUAGE: English

AB It has been demonstrated that parathyroid hormone can increase adenylate cyclase activity in the rat papilla, produce a small antidiuretic effect and in vitro can interfere with the action of arginine vasopressin on water transport. Clearance studies were performed in the anesthetized water diuretic thyroparathyroidectomized rat to evaluate further the effect of parathyroid hormone on urine concentration in the presence and absence

of arginine vasopressin. A maximal phosphaturic concentration of rat parathyroid hormone (2 µg/kg) reduced urine flow from 125 to 81 µL/min within 10 min. Addition of a maximal antidiuretic concentration of arginine vasopressin (100ng/kg) produced a delayed

and diminished antidiuretic response when compared with a group of rats not pretreated with parathyroid hormone (47 compared with 27 µL/min). However, a supramaximal arginine vasopressin concentration (1000ng/kg)

produced a

maximal antidiuretic effect in the presence of parathyroid hormone. To evaluate further the inhibitory effect of parathyroid hormone on arginine vasopressin-induced antidiuresis, parathyroid hormone (2µg/kg) was administered to one group of rats and a minimally effective arginine vasopressin concentration (7.5ng/kg) to another group, which produced a similar antidiuretic effect. However, the subsequent effect of a maximal antidiuretic arginine vasopressin concentration (100ng/kg) was again significantly blunted in the group pretreated with parathyroid hormone. Parathyroid hormone produced only a small increase in mean plasma calcium concentration, and glomerular filtration rate was not altered by either hormone. These results demonstrate that high physiol. concns. of parathyroid hormone do have a significant antidiuretic effect and can interfere with the action of arginine vasopressin. This suggests that parathyroid hormone may act as a partial agonist to arginine vasopressin in the collecting system.

L7 ANSWER 202 OF 221 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1993:219884 HCAPLUS

DOCUMENT NUMBER: 118:219884

TITLE: Pharmaceutical emulsion containing parathyroid hormone for pernasal administration

INVENTOR(S): Yamamoto, Nakayuki; Sugimoto, Michihiko; Morimoto, Seiki; Sakakibara, Hideo; Saita, Masaru; Shimozone,